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Occupational Criteria for
Chemical Agent VX

Final Report

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Rosemarie A. Faust
Dennis M. Opresko

Supported by
U.S. ARMY MEDICAL
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The U.S. Army has stockpiles of chemical agents that are or will be undergoing demilitarization; among these is the chemical agent VX. Military and civilian munition personnel involved in the demilitarization of VX will be potentially at risk from accidental exposures. In order to ensure the safety of these workers, the current standards must be reevaluated in light of the most recent toxicological data, using the most appropriate methodology for determining maximum safe exposure limits.

In this report, toxicological data for VX are reviewed to determine the most significant toxicological effects for defining minimum and no-effect levels; the basis for the current occupational standard is discussed; and a standard for VX is derived from both human and animal data correlating VX exposure to the inhibition of erythrocyte acetylcholinesterase. The current occupational exposure standard of 0.00001 mg/m^3 (8 hr TWA) is considered to be valid.

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OCCUPATIONAL CRITERIA FOR
CHEMICAL AGENT VX

FINAL REPORT

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Dennis M. Opresko

Chemical Effects Information Task Group
Information Research and Analysis Section
Health and Safety Research Division

SUPPORTED BY

U. S. ARMY MEDICAL
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EXECUTIVE SUMMARY

The U.S. Army has stockpiles of the chemical agent VX that require demilitarization. Military and civilian munition personnel involved in the demilitarization of this chemical will be potentially at risk from accidental exposures. In this report the general toxicology of VX is reviewed, current standards are reevaluated in light of the most recent toxicological data, and an occupational standard is recalculated from both human and animal data correlating VX exposure to the inhibition of red blood cell acetylcholinesterase (RBC-AChE).

1. General Toxicology

VX [S-(2-diisopropylaminoethyl) O-ethyl methylphosphonothiolate] is a potent anticholinesterase organophosphate agent. The chemical has a relatively low volatility and can produce toxic effects by absorption through the skin and conjunctiva of the eye, by inhalation, by ingestion, or by parenteral routes of administration. In the occupational setting, VX is considered primarily a contact and inhalation hazard. Some of the toxic symptoms in man induced by VX are miosis, headaches, nausea and vomiting, tightness of chest and cough, and increased sweating, lacrimation, and salivation, tension and jitteriness, tremors, and convulsions. Compared with human exposure estimates for the agent GB, VX is estimated to be twice as toxic by inhalation, 10 times as toxic by ingestion, and 170 times as toxic following dermal exposure.

VX is a toxic substance which can be lethal at very low doses. The estimated lethal median effective dose in humans is 7.5 μg/kg, equivalent to an ECt_{50} (concentration multiplied by exposure time) of 35 mg-min/m^3. LD_{50} values have been reported for several animal species and exposure routes and are consistently very low.

The principal mechanism by which VX induces acute toxicity is by inhibition of the enzyme acetylcholinesterase (AChE) and the subsequent rapid accumulation of acetylcholine at cholinergic synapses. Without AChE to inactivate acetylcholine, continuous stimulation of nerve fibers leads to disruption of nerve and organ function, leading to convulsions and death due to paralysis of the respiratory center. The symptoms of acute toxicity can be correlated with the inactivation of AChE within the neuroeffector junction or the effector itself. However, VX can apparently also affect nerve-impulse transmission by more direct processes as well. VX has been shown to directly affect the receptor sites for acetylcholine in excitable tissue by reacting with the receptor in a similar manner as acetylcholine.

VX also inhibits the chemically identical AChE which is present in red blood cells (RBC) and to a much lesser degree a second cholinesterase (pseudocholinesterase or plasma cholinesterase) which is present in blood plasma and some organs. Changes in RBC-AChE activity have been used as a monitor for VX exposure as well as an indicator of potential toxic effects. VX inhibited RBC-AChE ages slowly to a nonreactivatable form with a half-time of about 60 hr. Recovery from the effects of VX

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poisoning may occur through de novo synthesis of AChE and/or spontaneous reactivation of inhibited enzyme. Spontaneous reactivation of VX-inhibited RBC-AChE has been reported to occur at a rate of about 1%/hr.

Symptoms of VX toxicity have been correlated with RBC-AChE inhibition in studies with volunteers, who were given low levels of VX via intravenous, percutaneous, or oral routes of exposure. The clinical data indicate that toxic symptoms appear only when the RBC-AChE activity is reduced 40% or more (≤60% of baseline values). Performance-degrading effects are not expected to occur until blood enzyme activity falls to 70% below normal. Because blood levels of cholinesterase vary widely from individual to individual, pre-exposure baseline data are needed for comparison with postexposure levels.

Epidemiological and laboratory studies indicate that VX does not produce carcinogenic, mutagenic, or teratogenic/reproductive effects, and unlike some other organophosphates, VX has not been associated with delayed neuropathy. Animal studies indicate that subchronic exposures do not produce systemic effects other than those on the nervous system.

2. Basis for the Current Standard

The current DOD occupational exposure standard for VX is based on studies reported on in 1973, in which two toxicity endpoints were used: miosis (pupillary constriction) due to direct eye contact with VX and inhibition of blood cholinesterase following systemic absorption of VX.

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The VX exposure standard based on miosis was derived from extrapolations of miosis data for VX and GB, and from the rate of recovery of plasma cholinesterase which was used as a measure of the rate of recovery from miosis. VX was reported to be 25.6 times more potent than GB in producing miosis (in rabbits), while the recovery rate (in humans) was estimated to be 4 times faster, indicating that the no-effect concentration for VX would be 4/25 of the no-effect concentration for GB, or about 0.000017 mg/m^3.

The exposure standard for systemically absorbed VX was based on the maximum level of exposure resulting in no effect on RBC-AChE, i.e., the level producing no incidents of enzyme inhibition outside the 99% confidence range ($\pm$ 12.5% RBC-AChE depression) for random fluctuations in a normal unexposed population. This acute "no-effect" dose of 0.03 μg/kg was adjusted to avoid potential cumulative effects (i.e., increases in RBC-AChE inhibition during low level chronic exposures) by assuming that recovery from toxicity paralleled recovery from enzyme inhibition. A dose-effect accumulation factor of about 18 was calculated from experimentally derived rates of enzyme recovery and from the use of a mathematical model for dose-effect accumulation. The resulting chronic no-effect dose was 0.00168 μg/kg (0.00783 mg-min/m^3); equivalent to 0.0000163 mg/m^3 for a daily 8-hr exposure.

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Since the no-effect doses for miosis and AChE inhibition were about the same (0.000017 mg/m^3), an occupational exposure standard of 0.00001 mg/m^3 (1/10 the GB standard of 0.0001 mg/m^3) was proposed for VX, and this is the current DOD standard.

3. Derivation of an Occupational Standard for VX

In this report, an occupational standard for VX was derived from both human and animal data correlating VX exposure to the inhibition of RBC-AChE. Experimental human data indicate a dose-dependent decrease in RBC-AChE activity as expressed by:

$$y = 57.6 - 188.7 \log x \quad (r = -0.71)$$

where: x = intravenous dose in μg/kg
  y = RBC-AChE activity (as % of baseline)

Clinical data have shown that for single acute exposures to VX, symptoms of toxicity appear only when RBC-AChE activity is reduced 40% or more ($\leq 60\%$ of baseline value). For exposures to the other cholinesterase inhibitors, a 30% reduction in enzyme activity has been recommended as the action level for removing workers from exposure. In the absence of more specific experimental data, a 30% level of RBC-AChE inhibition can be treated as a potential LOAEL (lowest-observed-adverse-effect level) for VX. Levels for enzyme inhibition less than 30% are not likely to cause adverse effects. A NOAEL (no-observed-adverse-effect level) might be selected at 15% (85% of baseline) to allow for a 15% range in variability in individual responses. This ED$_{15}$ is equivalent to a dose of 0.72 μg/kg.

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Using 0.72 μg/kg as a NOAEL, as well as an uncertainty factor of 18 to adjust for possible cumulative effects, and an uncertainty factor of 10 to allow for potential individual variability in sensitivity to VX, an air concentration of 0.000013 mg/m^3 was calculated to be an acceptable occupational exposure standard.

For animals, the relationship between VX dose (subcutaneous) and RBC-AChE activity can be expressed by the formula:

$$y = 52 - 74 \log x \quad (r = -0.976)$$

where: x = subcutaneous dose in μg/kg
        y = RBC-AChE activity (as % of baseline)

Subchronic studies on rats injected subcutaneously once per day, 5 days per week, have shown that cumulative effects (i.e., increased RBC-AChE inhibition) occurred at 7, 14, and 30 days, but generally not at 60 and 90 days. The response at 30 days was used as a measure of the maximum cumulative effect, and the dose-response relationship at 30 days expressed by the formula:

$$y = 25 - 33 \log x \quad (r = -0.979)$$

where: x = subcutaneous dose in μg/kg
        y = RBC-AChE activity (as % of baseline)

The dose producing a 15% depression of RBC-AChE at 30 days is equivalent to about 0.015 μg/kg. Using this value as a NOAEL, an occupational exposure standard was then calculated by using an interspecies body size adjustment factor of 0.179 and an uncertainty factor of 10 to allow for potential individual variability in sensitivity to VX. The resulting

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standard for an 8-hr exposure would be 0.0000011 mg/m^3. This value is smaller than that derived from the human data and implies a greater sensitivity of rats to VX. This may be explained in part by differences in RBC-AChE activity between the two species. In rats RBC-AChE activity has been reported to be about $1/8^{th}$ that of humans; therefore, the same dose would produce 8 times the effect.

In spite of the differences between the endpoints and the uncertainty factors used in this report and those used to derive the current DOD occupational exposure standard for VX, the final calculated values (based on the human data) are almost identical, and, therefore, the current standard of 0.00001 mg/m^3 is considered to be valid.

ACKNOWLEDGMENTS

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1. INTRODUCTION

The U.S. Army has stockpiles of chemical agents that require demilit-
arization; among these is the nerve agent VX [S-(2-diisopropylaminoethyl)
O-ethyl methylphosphonothiolate], a potent anticholinesterase organophos-
phate agent. Other chemical agents scheduled for demilitarization are the
G agents which include GA (tabun), GB (sarin), and GD (soman). It is
estimated that between 1961 and 1967, approximately 5,000 tons of VX have
been produced and stockpiled in the United States (Meselson and Robinson
1980; Koelle 1981). Military and civilian munition personnel involved in
the demilitarization of VX will be potentially at risk from accidental
exposures. In addition, VX is used in various laboratories for research
purposes. The potential therefore exists for chronic as well as acute
exposure of personnel during demilitarization, transportation, research,
and disposal, with dermal and inhalation exposure being the most probable
routes of concern. In order to ensure the safety of these workers,
current standards must be reevaluated in light of the most recent toxico-
logical data, using the most appropriate methodology available for
determining maximum safe exposure limits.

Currently accepted and proposed methodologies for setting exposure
standards are generally similar in that they identify two separate
categories of toxic chemicals: noncarcinogenic substances that have a
toxicity threshold and carcinogenic substances that do not have a thresh-
old. The organophosphate nerve agents fall into the category of threshold
toxicants. Occupational exposure limits of VX should therefore be based

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on an evaluation of the lowest observable adverse effect and the direct or indirect determination of the maximum chronic exposure level which could be tolerated without producing that effect. For toxicity data derived from animal studies, equivalent human dose levels have to be calculated or extrapolated. Species differences in metabolic rates, respiratory parameters, and pulmonary and gastrointestinal absorption have to be considered. For human as well as animal studies, the data must also be adjusted to reflect the expected exposure conditions (exposure route, duration, frequency, and total exposure period). Such extrapolations are based on empirically derived data and on dose/response relationships. Additional uncertainty or safety factors are also used to adjust for potential variables which cannot be determined experimentally (e.g., sensitive subpopulations) (Opresko 1987).

2. CHEMICAL AND PHYSICAL PROPERTIES

The chemical agents considered for demilitarization by the U.S. Army are organophosphate compounds usually containing a fluorine, sulfur, or cyanide substituent group. They fall into two categories: the G agents which are derivatives of phosphoramidocyanidic acid or methylphosphono-fluoridic acid, i.e., they have cyano or fluoro groups attached to phosphorus, and the V agents which are derivatives of methylphosphono-thioic acid, i.e., they have sulfur attached to the phosphorus of the molecule (Dacre 1984).

The chemical names (10th C.I. of Chemical Abstracts) and identification numbers for agents VX, GA, GD, and GB are:

1. Agent VX: (CAS No. 50782-69-9; Edgewood Arsenal No. 1701) Phosphonothioic acid, methyl-, S-[2-[bis(1-methylethyl) amino]ethyl] O-ethyl ester

2. Agent GA (tabun): (CAS No. 77-81-6; Edgewood Arsenal No. 1205) Phosphoramidocyanidic acid, dimethyl-, ethyl ester

3. Agent GB (sarin): (CAS No. 107-44-8; Edgewood Arsenal No. 1208) Phosphonofluoridic acid, methyl-, methylethyl ester

4. Agent GD (soman): (CAS No. 96-64-0; Edgewood Arsenal No. 1210) Phosphonofluoridic acid methyl-, 1,2,2-trimethylpropyl ester

Specific physical and chemical properties of VX are as follows:

Structural Formula:

$$H_3C-\underset{\underset{O-CH_2CH_3}{|}}{\overset{\overset{O}{\|}}{P}}-S-CH_2CH_2-N\overset{CH(CH_3)_2}{\underset{CH(CH_3)_2}{}}$$
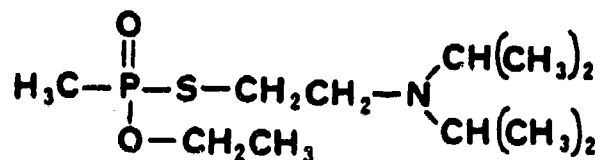
Molecular Formula:	$C_{11}H_{26}NO_2PS$
Molecular Weight:	267.37
Physical State:	Straw-colored, oily liquid (NRC 1984)
Boiling Point:	298°C (Harris et al. 1978)
Freezing Point:	Below -51°C (Harris et al. 1978)
Solubility:	Miscible with water (Crabtree and Sarver 1977); readily soluble in all organic solvents (Goldman et al. 1987)
Density:	1.0083 at 25°C (Harris et al. 1978)
Vapor Pressure:	0.00066 mm Hg at 20°C (NRC 1984)
Vapor Density:	9.2 (compared to air) (U.S. Army 1973)
Flash Point:	159°C (Kaye 1983)
Volatility:	10.5 mg/m^3 at 25°C (Harris et al. 1978)
Odor:	Odorless (Windhoz et al. 1983)
Viscosity:	1.4777 centistokes at 25°C (Goldman et al. 1987)
pKa:	7.9 (Windholz et al. 1983)
Stability:	Combustion will release SO_x, NO_x, and P_2O_5 (Ottinger et al. 1973). Vx is stabilized with N,N'-diisopropyl-carbodiimide to prevent hydrolytic degradation (U.S. Army 1964).

3. GENERAL TOXICOLOGY

Several general reviews of the health and environmental effects of nerve agents and related compounds (organophosphate insecticides) are available (O'Brien 1960; Dacre 1984; Matsumara 1976; NRC 1982; Carnes et al. 1986). Ross et al. (1983) prepared a comprehensive review on the toxicology and health effects of VX. A toxicity evaluation of chemical agents and their breakdown products in support of the Chemical Stockpile Disposal Program has recently been published (Watson et al. 1988).

In this report pertinent toxicological data are reviewed to determine the most significant health effects for defining minimum and no-effect levels of VX. Some of the studies conducted in the 1960s as part of the Edgewood Arsenal military testing program are reviewed, as well as recent data from a broad-based toxicological evaluation of the effects of VX in experimental animals conducted by investigators at the University of California, Davis (Goldman et al. 1987) for the U.S. Army Medical Research and Development Command. No clinical work with VX has been conducted at Edgewood Arsenal since 1967 (Sidell and Groff 1974), but a recent account (Mason 1987) indicates that British scientists currently are testing chemical agents on members of the armed forces at the Chemical Defense Establishment at Porton Down, Wiltshire. One series of tests reportedly is designed to determine levels at which soldiers can function effectively despite the presence of chemical agents in the atmosphere. The article does not indicate whether VX is one of the chemicals being used for human trials.

3.1 TOXICOKINETICS

The absorption, distribution, metabolism, and excretion of or-
ganophosphate compounds that inactivate the enzyme acetylcholinesterase
(AChE) have been reviewed in a report prepared by the Committee of
Toxicology of the National Research Council (NRC 1982). According to this
report, the commonly encountered organophosphate anticholinesterase agents
are lipid-soluble, and this property accounts for a rapid and effective
absorption by almost any route of exposure. Once absorbed, these com-
pounds or their metabolites bind to proteins in the blood and tissues (NRC
1982). However, there is no evidence to suggest that this mechanism plays
a significant role in modifying the effects of acutely toxic concentra-
tions of these compounds (McNamara et al. 1973).

Little is known about the metabolism and excretion of VX. The
chemical, a derivative of methylphosphonothioic acid, is a relatively
nonvolatile, rapidly acting, lethal nerve agent. Because of its low
volatility, the hazard from VX is primarily that of absorption through the
skin, although it can be absorbed by inhalation of vapor or aerosol, or
through the gastrointestinal tract by ingestion (Ottinger et al. 1973;
U.S. Army 1980). Following percutaneous exposure, VX slowly penetrates
the skin, undergoing virtually no degradation (van Hooidonk et al. 1980).
In vitro studies suggest that VX can penetrate the skin in unaltered form,
pass through the epidermis and dermis, reach the nerve membranes, and
accumulate within the nerve cells (Farquharson et al. 1980).

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When VX is absorbed into the body, some of it combines rapidly with AChE, other esterases, and possibly with other proteins. AChE and similar cholinesterases, such as butyryl cholinesterase (pseudocholinesterase), are found in muscle tissue and blood, as well as in the nervous system. The rate of reaction of VX with AChE in human red blood cells (RBC-AChE) is rapid, as evidenced by its bimolecular rate constant (k_i) of 1.6×10^7 L mole^{-1} min^{-1} (McNamara et al. 1973).

3.2 ACUTE TOXICITY

A survey of the acute toxicity data for VX and other chemical agents indicate that they are among the most toxic substances known to man. The principal toxic effect of VX is inhibition of AChE resulting in a buildup of acetylcholine at cholinergic synapses. Without AChE to inactivate acetylcholine, overstimulation of nerve fibers leads to a disruption of nerve and organ function. In comparison to the G nerve agents, VX is more toxic than either GB (sarin) or GA (tabun). In humans, it is approximately twice as toxic as GB by inhalation, 10 times as toxic by oral administration, and approximately 170 times as toxic following percutaneous administration (NRC 1984).

3.2.1 Symptoms

Initial symptoms of poisoning by VX may occur within several minutes of exposure, but may be delayed as long as 30 minutes in the case of local effects or up to 2 hours for systemic effects. Exposure to VX vapor

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initially produces local effects involving the eyes and respiratory tract. The following symptoms may be evident: miosis (may be unequal), frontal headaches, eye pain on focusing, dimness of vision, occasional nausea and vomiting, and hyperemia of the conjunctiva. Respiratory symptoms include rhinorrhea, tightness in the chest, cough, increased bronchial secretion, dyspnea, pulmonary edema, cyanosis, and respiratory arrest leading to death. Increased sweating, lacrimation, and salivation may result from minimal local exposure or more severe systemic exposure. Also present may be slight bradycardia, blurring of vision, increased urinary frequency, and involuntary micturition. Effects on the central nervous system in mild exposures may include tension, anxiety, jitteriness, restlessness, insomnia, or excessive dreaming. Higher exposures may cause headache, tremors, drowsiness, difficulty in concentrating, impairment of memory, apathy, withdrawal, and depression. With the appearance of moderate symptoms, abnormalities in the electroencephalogram (EEG) may occur which may precipitate convulsions (U.S. Army 1980).

3.2.2 Lethality

VX is a very toxic substance which can cause death at very low doses. It is both an inhalation and a skin contact hazard. Despite its low vapor pressure, a person would have to breathe air saturated with VX vapor for only a few minutes to attain the LCt_{50} (NRC 1984). According to McNamara et al. (1973), the estimated lethal median effective dose in humans is 7.5 μg/kg, equivalent to an ECt_{50} (concentration multiplied by exposure time) of 35 mg-min/m^3 for exposures of 20 sec to 2 min. The no-death level was

calculated to be 0.94 μg/kg or 4.4 mg-min/m^3. The authors note that the terms used to describe the lethal/toxic response for humans are derived values and did not involve human experimentation. The values therefore should be viewed only as indicators of the general magnitude of toxicity for establishing exposure limits.

In a tabulation of VX toxicity data by Watson et al. (1988), the LCt$_{50}$ for mildly active humans was reported to range from 20 to 50 mg-min/m^3. The Army Dispersion Code (D2PC) values for 1% lethality and no deaths are 4 mg-min/m^3 and 2 mg-min/m^3, respectively. Watson et al. (1988) considered the no-death level for susceptible subpopulations (infants and elderly) to be 20% of the D2PC code for no death (i.e., 20% of 2 mg-min/m^3) or 0.4 mg-min/m^3.

Intravenous studies of VX in man showed that doses up to 2.12 μg/kg administered over a 5.5 hr period were not lethal (Kimura et al. 1960).

The acute toxicity of liquid VX as well as VX aerosols has been assessed in a number of animal species by various routes of admini-stration. Clinical signs of acute toxicity are general neuromuscular weakness, tremors, twitching, seizures, miosis, hypersalivation, and respiratory distress. VX also appears to have a direct effect on the heart, producing significant changes in ventricular function (Robineau and Guittin 1987). Compared with the G agents, VX is slower in producing symptoms of acute toxicity (Rickett et al. 1986). In all cases, the

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ultimate cause of death is respiratory failure (Crook et al. 1983; Haggerty and Kurtz 1985; Manthei et al. 1985; Rickett et al. 1986).

LD_{50} values, summarized in Tables 1 and 2, have been reported for several species and exposure routes, and are consistently very low. For example, by the percutaneous route, the $LD_{50}s$ range between 0.05 mg/kg for the mouse to 0.4 mg/kg for the pig. A comparison of percutaneous and intravenous $LD_{50}s$ in different species shows that the skin $LD_{50}s$ are higher, suggesting that only a fraction of the VX applied reaches the bloodstream. For inhalation exposures, reported $LCt_{50}s$ range between 7 and 50 mg-min/m^3. A range of $LCt_{50}s$ between 3.5 and 180 mg-min/m^3 has been reported for percutaneous exposures to aerosols (Watson et al. 1988). Inhalation studies with laboratory animals indicate that VX is equally toxic in vapor or dispersed aerosol form. In the mouse, for example, the LCt_{50} is 4.0 mg-min/m^3 for vapor exposure and 7.0 mg-min/m^3 for aerosol exposure (Crook et al. 1983).

In experimental animals, there appears to be a poor correlation between the lethal effects of VX and inhibition of cholinesterase (ChE) in blood or tissues (Ellin 1981; Wills 1972). In cats, for example, no correlation was found between the levels of RBC-AChE and survival after intravenous injection with lethal doses of VX (Marzulli et al. 1960). Wills (1972) reports that although there is a poor correlation between intravenous lethality and enzyme inhibition, the best correlation was found between the inhibition of ChE in the bulbar region of the brain and RBC-ChE.

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TABLE 1. TOXICITY OF LIQUID VX TO SEVERAL ANIMAL SPECIES
BY THE INTRAVENOUS AND PERCUTANEOUS ROUTES

| | LD_{50} (mg/kg) | |
Species	Intravenous route	Percutaneous route
Mouse	0.014	0.046
Rat	0.008	0.10
Guinea Pig	-	0.035
Rabbit	0.008	0.025
Dog	0.006	0.054
Monkey	0.008	ca. 0.008
Goat	<0.005	ca. 0.020
Cat	ca. 0.003	0.122
Pig	0.009	0.40

Source: Crook et al. 1983. Animals were shaved and depilated prior to percutaneous exposure.

TABLE 2. TOXICITY OF LIQUID VX TO RODENTS AND RABBITS BY VARIOUS
ROUTES

| | LD_{50} (mg/kg) | | |
Route	Mouse	Rat	Rabbit
Intravenous	0.014	0.008	0.008
Intramuscular	0.017	0.014	ca. 0.009
Subcutaneous	0.016	0.015	-
Intraperitoneal	0.038	0.046	-
Intragastric	-	0.100	-
Percutaneous	0.046	0.10	0.025

Source: Crook et al. 1983

3.2.3 Mechanism of Action

3.2.3.1 Cholinesterase Inhibition

The toxicity of organophosphate nerve agents is attributed to their inhibition of enzymes located in the nervous system. In the case of acute neurotoxic effects, the organophosphates act as inhibitors of acetylcholinesterase (AChE), the enzyme responsible for deactivating the neurotransmitter acetylcholine at nerve-nerve and nerve-muscle junctions. AChE has two active sites on the molecule, the esteratic site and the anionic site. Normal inactivation of acetylcholine involves the initial coulombic binding of the acyl carbon to the esteratic site and the cationic nitrogen group to the anionic site. This is followed by an acylation reaction in which hydrogen is transferred from the enzyme to the choline moiety of the acetylcholine. The acylated complex is hydroxylated by reaction with water and then rapidly deacylated by transfer of hydrogen to the enzyme. The regenerated enzyme is thus continuously available for controlling acetylcholine levels (Wilson and Bergman 1950; Opresko 1987).

Organophosphate compounds function as substitute substrates for AChE, in effect taking the place of acetylcholine. The phosphorus atom binds at the esteratic site in a process analogous to acylation. This reaction is called phosphorylation. Dephosphorylation, or reactivation of the enzyme, occurs through hydrolysis by water or other nucleophilic agents. Dephosphorylation proceeds at a much slower rate than deacylation; consequently,

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there is a depletion of active cholinesterase and a buildup of acetyl-
choline in the nervous system (Matsumara 1976).

The effectiveness of organophosphates as AChE inhibitors depends on
how strong an affinity the phosphorus moiety has for the esteratic site on
the enzyme and also how slowly dephosphorylation occurs. Phosphorylation
is a function of the electrophilicity of the phosphorus group, and any
chemical substituents attached to the phosphorus which increase the
electrophilicity will increase the anticholinesterase activity (Matsumara
1976). The rate of reactivation, or recovery, of the enzyme varies with
the complexity and basicity of the alkylphosphate portion of the or-
ganophosphate (Matsumara 1976). Aging, the conversion of inhibited AChE
over a period of time into a form that cannot be reactivated, results from
the release of an alkyl group from the alkoxy group on the phosphorus
attached to the enzyme. Aging and reactivation are interrelated, i.e., to
demonstrate that aging takes place, one shows that reactivation does not
occur (Ellin 1981). Binding of AChE with most organophosphate agents is
essentially irreversible since the rate of spontaneous regeneration of
inhibited enzyme is quite low (NRC 1982).

Anticholinesterase activity of organophosphates can be indicated
by either the bimolecular rate constant (K_i) for the reaction of the
organophosphate compound with the enzyme or by the median inhibition
concentration (I_{50}), which results in 50 percent inhibition of the enzyme.
The relationship between the I_{50} value and k_i is expressed by the follow-
ing equation: $k_i = 0.695/I_{50}t$ (Opresko 1987).

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3.2.3.2 Direct Neurotoxicity

Although the inhibition of AChE within neuroeffector junctions or the effector itself is thought to be responsible for the major toxic effects of organophosphorus chemical agents, these compounds can apparently affect nerve-impulse transmission by more direct processes as well. Recent technological advances have provided the means of identifying the compounds' direct action on excitable tissues, receptors, and ionic channels. In addition to AChE inhibition, VX reacts directly with AChE receptors and other neurotransmitter receptors (Chen and Chi 1986; Idriss et al. 1986). Albuquerque et al. (1985) have shown that GA, GB, and GD are capable of changing receptor sites in a manner similar to that produced by acetylcholine, which promotes the conductance of electrophysiological signals associated with stimulation of neuromuscular function. VX, on the other hand, prevents the signal conductance, even if acetylcholine binds to the receptor, thereby interrupting neuromuscular function. Rickett et al. (1987) indicate that a growing recognition of the significance of the direct action on nerve-impulse transmission as opposed to enzymatic effects is causing a re-examination of theoretical assumptions concerning the physiological consequences of nerve agents. In the meanwhile, however, existing data which focus on the relationship of AChE inhibition or other effects, such as miosis, and toxicity must be relied on to evaluate the potential adverse health effects used in the derivation of occupational exposure standards.

3.2.4 Blood Cholinesterases

Even though the toxicity of organophosphate agents appears to be
correlated to the inactivation of AChE in nerve tissues, the compounds
also react with a chemically identical AChE that is bound to the surface
of erythrocytes (RBC-AChE), and with a second cholinesterase (termed
pseudocholinesterase, plasma cholinesterase, or butyrylcholinesterase)
which is found in blood plasma and serum, and in some organs, such as
liver. Both can be inactivated by organophosphate compounds. Changes in
the activity levels of these blood cholinesterases have been used to
monitor exposure to organophosphate nerve agents as well as to indicate
potential toxicity.

According to Eto (1974), the two cholinesterases are distinguished
by their substrate specificity: AChE hydrolyzes its natural substrate,
acetylcholine, while pseudocholinesterase preferentially hydrolyzes
butyryl and propionylcholines. The biological function of pseudocholin-
esterase has not been elucidated. Complete inactivation of the enzyme
apparently produces no detectable adverse effects (Koelle 1981), and it
has been estimated that 3 of 1,000 individuals may lack pseudocholin-
esterase (Wagner 1983; Wills 1972). Pseudocholinesterase reportedly can
be depressed by a variety of conditions unrelated to exposure to anti-
cholinesterase agents. They include, but probably are not limited to,
liver disease, heart disease, allergies, neoplasms, and pregnancy (Wills
1972).

Wills (1972) indicates that the AChE of red blood cells does nothing more than control the cells' permeability. He suggests that both RBC-AChE and pseudo-ChE exert a protective action with respect to functional AChE by reacting with some of an absorbed anticholinesterase compound before it reaches the functional AChE in the tissues. The protective action will vary, depending on the physical properties and chemical stability of the enzyme inhibitor. After inactivation by an irreversible inhibitor, the ChE activity of plasma is replaced more rapidly than that of erythrocytes.

The RBC-AChE activity is probably reduced to a greater extent than the functional AChE in nerve tissues, particularly if an anticholinesterase compound is administered gradually. In studies with GB, Grob and Harvey (1953, 1958) found that repeated oral administration to volunteers over several days would gradually reduce the RBC-AChE activity to near zero without concomitant symptoms of toxicity. Ellin (1981) reported that if an organophosphorus agent is administered to experimental animals over a long period of time, the blood levels of cholinesterase activity can drop to near zero, yet the animals survive. However, if blood levels drop to zero rapidly, the animal dies. A review of the available literature indicates that cholinesterase levels in tissues cannot be depressed to levels lower than those in red blood cells.

Blood cholinesterase activity levels may vary between individuals and between species. In a small population (22 subjects) tested biweekly throughout one year, Sidell and Kaminskis (1975) found no correlation between RBC-ChE activity and age, nor was there a seasonal variation in

enzyme activity. There was a significant difference in the plasma ChE activity in men and women (4.45 versus 3.75 μmoles/min/mL, p <0.01). The levels of RBC-AChE were more constant than those of plasma-ChE. The highest coefficient of variation of RBC-AChE was 4.1%/single subject; the average range of variation was ± 2.1% for men and ± 3.1% for women.

Table 3 shows that RBC-AChE activities as well as optimum substrate concentrations vary considerably between species (Ellin 1981). The monkey appears to have properties most similar to humans, while the RBC-AChE activity in rats is about a magnitude lower than that in humans. A literature survey of normal values for ChE activity in erythrocytes and plasma (Wills 1972) indicates that both the plasma- and RBC-AChE activity are higher in human males than in females. The reverse is true for rats, with higher levels in females than in males.

3.2.5 Cholinesterase Inhibition by VX

VX reacts very specifically with AChE from various sources (Goosens et al. 1984; Goudou and Rieger 1983; Vigny et al. 1978) with an I_{50} of 5 x 10^{-1} M, a concentration many magnitudes less than necessary to inhibit non-specific cholinesterases (Goldman et al. 1987). In studies on cholinesterases extracted from mouse skeletal muscle, Goudou et al. (1983) found that the concentration of VX producing 50% inhibition of AChE was 4.1 x 10^{-10} M whereas the concentration needed for 50% inhibition of pseudocholinesterase was 1.1 x 10^{-8} M.

TABLE 3. ACETYLCHOLINESTERASE ACTIVITIES
IN VARIOUS ANIMAL SPECIES

Species	Red Blood Cell Activity[a]	Optimum Substrate Concentration[b] (M)
Human	12.6	2×10^{-3}
Monkey	7.1	2×10^{-3}
Pig	4.7	1×10^{-3}
Goat	4.0	2×10^{-3}
Sheep	2.9	2×10^{-3}
Mouse	2.4	2×10^{-3}
Dog	2.0	2×10^{-2}
Guinea pig	2.7	2×10^{-3}
Rabbit	1.7	5×10^{-3}
Rat	1.7	5×10^{-3}
Cat	1.5	5×10^{-3}

Source: Ellin 1981

[a]μmol/mL/min at optimum substrate concentration

[b]Acetylthiocholine iodide

A relatively small number of clinical studies with VX have been reported. The response to VX administered to volunteers was characterized in a series of experiments mostly conducted at Edgewood Arsenal, using sublethal doses of the agent via intravenous, percutaneous, or oral routes of exposure (Kimura et al. 1960; Sim and Stubbs 1960). Inhalation experiments apparently have not been attempted in humans, probably due to the technical complications and hazards involved.

The VX dose of concern is the dose present in the blood stream that can be correlated with the activity of AChE in the red blood cells. It has been established that the inhibition of cholinesterase at the neuro-effector junctions of tissues does not become significant and that adverse health effects do not develop until the enzyme activity is depressed below a certain level. Although cholinesterase activity in the central nervous system is probably the true measure of toxicity in man, there is no safe way to obtain samples for assay. Therefore, monitoring of cholinesterase activity in blood components serves as an alternate method.

VX is more toxic to humans when administered intravenously than when administered percutaneously or orally. An intravenous study conducted by Kimura et al. (1960) became an important point of reference for experiments and extrapolations performed in later studies. In this study, seven volunteers were injected intravenously with doses ranging from 0.04 to 2.12 μg/kg of VX either in 30-sec injections or by slow infusion. The following observations were made: 0.225 μg/kg of VX administered over 30 seconds resulted in minimal RBC-AChE depression; 1.0 μg/kg administered

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over 30 seconds resulted in an approximate 50% depression of RBC-AChE; and 2.12 µg/kg administered over 5.5 hr appeared to be the maximum tolerated dose not requiring atropine or oxime therapy and/or artificial resuscitation. RBC-AChE, which had been depressed to 35 to 50% of normal, spontaneously returned to 80 to 90% of normal within 14 days. According to McNamara et al. (1973), the data indicate that an intravenous dose of 0.1 µg/kg would not produce measurable RBC-AChE inhibition.

Correlating the signs and symptoms of VX poisoning with RBC-AChE inhibition, Kimura et al. (1960) found that when about 40% of RBC-AChE activity was inhibited, there was a temporary increase in the minute volume of respiration, headache, a slight decrease of systolic pressure, and flushing of the skin. At 65% inhibition there was perspiration, at 75% inhibition complaints of tiredness, and at 85% inhibition visual disturbances, profuse salivation, vomiting, and pallor.

In a later study performed in 1965 (and published in 1974 in the open literature), Sidell and Groff described the symptoms and cholinesterase activity in man after intravenous injection of VX in doses of 1.2, 1.3, 1.4, 1.5, 1.6, or 1.7 µg/kg. In general, there was a dose-dependent depression of RBC-AChE (Figure 1) (plasma-ChE activity was never depressed more than 20%); this depression was greatest one hour after VX injection. Spontaneous recovery of RBC-AChE activity began soon after maximum inhibition and proceeded at a rate of 1% per hour over 70 hours. Most of the subjects had a mild to moderate increase in heart rate and blood pressure the first 3 hours after injection of VX; however, these values

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RBC-AChE
(% baseline)

DOSE   ug/kg
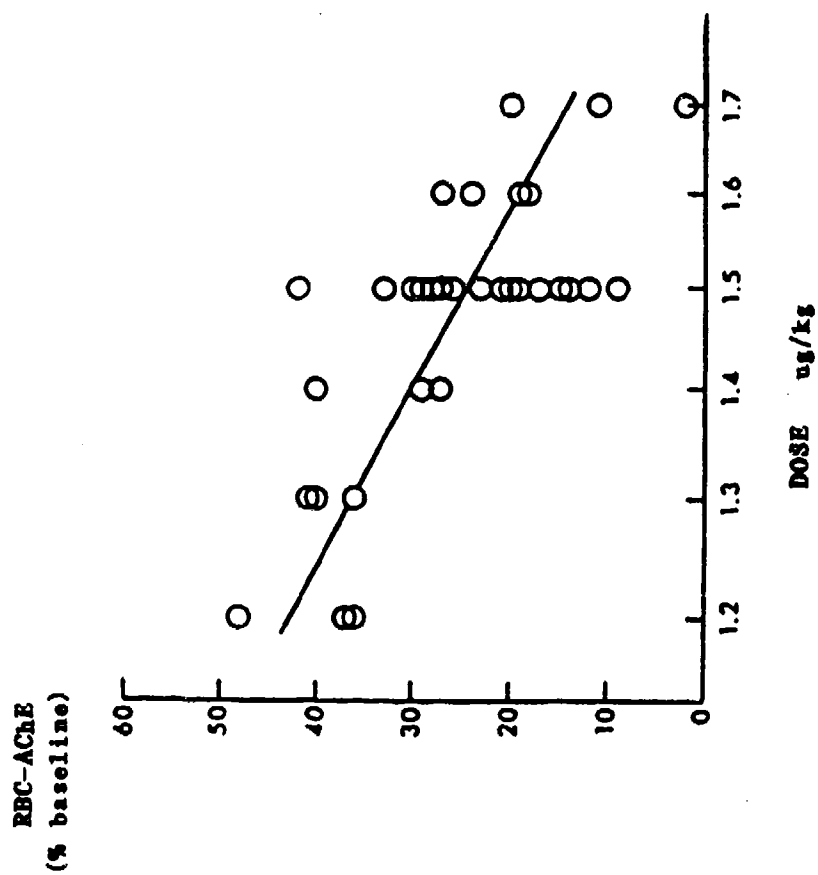
Figure 1. Relationship of VX exposure in humans vis single intravenous doses with the minimal measured RBC-AChE activity (as percent of baseline). $y = 57.6 - 188.7 \log x$; $r = -0.71$.

Source: Sidell and Groff (1974).

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did not differ significantly from control subjects. Of the 18 subjects injected with 1.5 μg/kg, 11 experienced dizziness, 4 transient nausea, and 6 vomited. The latter group had a mean minimal RBC-AChE value of 20%, while 7 subjects who were asymptomatic had a mean minimal value of 28%. Symptoms were maximal at one hour, which coincided with minimum cholinesterase values. The symptoms of VX poisoning related to RBC activity are listed in Table 4.

TABLE 4. SYMPTOMS OF VX POISONING RELATED TO RBC-AChE INHIBITION

% Inhibition	Symptoms
> 50%	Headaches that were rarely associated with measurable pupillary constriction
60 to 70%	Slight nausea and headache were common
70 to 80%	More severe nausea; abdominal cramps were often present; vomiting, weakness, photophobia, a feeling of "chilliness"
80 to 90%	Nausea, vomiting, abdominal cramps, and weakness became more severe and protracted; pupillary constriction and diarrhea in a few subjects

Source: Adapted from Sim and Stubbs (1960).

Sim and Stubbs (1960) performed a percutaneous dose-response study, in which VX was delivered to the surface of forearm skin either as a single drop or as multiple drops. Forty volunteers were treated with neat doses

of VX ranging from 5 to 35 μg/kg of body weight, and 12 subjects were given 20 μg/kg in multiple drops. Application of 35 μg/kg in a single drop produced an average fall of whole blood cholinesterase to 44% (range 9% to 77%) of pre-exposure values. In contrast, Kimura et al. (1960) have demonstrated that intravenous infusion of only 1 μg/kg produced a fall of whole blood cholinesterase to 43% of normal. Even though the percutaneous values were scattered over a wide range (9-77%) compared to the narrow range (38-46%) of the intravenous values, Sim and Stubbs (1960) suggested a percutaneous-to-intravenous ratio of Fig. 1 effectiveness of 1:33. A linear relationship between the percutaneous dose and the amount absorbed was also suggested. Dose-response curves were computed for the neat agent, and the doses required to produce 50% reduction in cholinesterase in red blood cells, plasma, and whole blood were predicted (Table 5). Sim and Stubbs (1960) observed that VX poisoning rarely occurred prior to 6 hours after VX application, and that the more rapid the fall of cholinesterase levels, the shorter the time between application of the agent and onset of symptoms.

TABLE 5. PREDICTED AMOUNTS OF PERCUTANEOUSLY ADMINISTERED VX REQUIRED FOR ChE_{50}

Time Period	RBC	Plasma	Whole Blood
12 hr	34 μg/kg	54 μg/kg	36 μg/kg
24 hr	29 μg/kg	52 μg/kg	37 μg/kg

Source: Adapted from Sim and Stubbs (1960).

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Using percutaneous data from human experiments (Sim and Stubbs 1960; Sim 1962), Feinsilver et al. (1965) calculated correlation coefficients between ChE activity of whole blood, red blood cells, and plasma following VX exposure. The best correlation was found between the whole blood-ChE and RBC-ChE at the dose level of 25 μg/kg. They further calculated that the magnitude and time of occurrence of the minimum ChE activity was dependent on size of dose as well as site of application. As the dose of VX increased, the whole blood-ChE, RBC-ChE, and plasma-ChE decreased; the RBC-ChE being the most, and plasma-ChE the least affected.

Sim et al. (1964) conducted experiments in which 16 military personnel volunteered to drink 2 L of water containing VX, each day for a 7-day period (administered in 4 doses per day at 2-hr intervals). The daily dose of VX was 1.43 μg/kg (100 μg/70 kg individual). None of the individuals exhibited any noticeable signs or symptoms of poisoning, but their mean RBC-AChE activity was depressed about 60%, i.e., the enzyme activity was reduced to 40% of pre-exposure levels at the end of the treatment period. The amount of RBC-AChE inactivated after each successive dose decreased progressively over the 7-day experimental period.

In the Sidell and Groff (1974) study, some individuals also received oral doses of VX. Only 4 of 32 exhibited symptoms of toxicity, even though their RBC-AChE levels were greatly depressed. The dose inhibiting RBC-AChE by 50% for oral VX administration was calculated as 2.3 μg/kg. Maximum AChE depression occurred about 2.5 hr after oral administration compared to 1 hr after iv treatment. The data also indicated that

VX-inhibited RBC-AChE undergoes spontaneous reactivation at a rate of about 1%/hr. The VX-inhibited RBC-AChE "ages" (dealkylates) to a non-reactivable form with a half-time of about 60 hr, considerably slower than other chemical agents (McNamara et al. 1973; Craig et al. 1977).

3.2.6 Recovery of Cholinesterase Activity

After depression by VX, the recovery of both RBC- and plasma-ChE activity in man is initially rapid followed by a slower phase. Using data from six human subjects, McNamara et al. (1973) derived a recovery constant of 0.396/day (39.6%/day) for plasma-ChE. Using data from one subject, plasma-ChE activity returned to normal after 12 days, whereas RBC-AChE activity was 70 to 80% of normal (based on different measurement methods). They note that although RBC-AChE levels recover at a slower rate after the 12th day, i.e., at a rate of about 0.8%/day, the rapid recovery phase includes more than half of the total recovery. According to these investigators, the initial recovery probably reflects a dephosphorylation reaction at the esteratic site of the enzyme while the slow component of recovery represents the inhibited RBC fraction whose enzyme had undergone aging by VX (see also Section 5.4.2). McNamara et al. (1973) postulated that the observed recovery may be a combination of processes, i.e., de novo synthesis of cholinesterase, conversion of enzyme from the inactive precursor form, or spontaneous reactivation of agent-deactivated enzyme, and others. RBC-AChE recovery was thought to be a combined zero-order and pseudo first-order process.

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Spontaneous recovery of ChE levels were also demonstrated in per-
cutaneous studies. Sim and Stubbs (1960) noted a spontaneous recovery of
plasma-ChE levels in subjects 12 hours after percutaneous exposure to VX.
By 48 hours, values for all blood components had recovered. In another
study, Sim (1962) observed that six subjects treated on the forehead with
10 μg/kg of VX (without drug therapy) had a rapid spontaneous recovery of
RBC-AChE activity during the first 4 days after treatment and then a more
gradual recovery after that. Cholinesterase levels were 20-55% of normal
at the time of maximum depression, which was usually about 4 hours after
application of VX. Thirty two subjects who exhibited symptoms (weakness,
fasciculations, slurring of speech, diplopia, and nausea) and had ChE
levels of 31% below normal, or below, were given an immediate oxime
treatment. The symptoms generally subsided within 30 to 60 minutes after
oxime administration and did not return. The study also showed that there
is a difference in sensitivity to penetration by VX of various body sites,
the head and neck areas being most sensitive. Another observation
indicated that rapid percutaneous penetration of VX with a resultant rapid
fall in ChE activity was associated with more severe symptoms than slow
penetration, even though the drop in ChE was almost the same.

As was noted in humans, experimental animals also display a biphasic
recovery of blood cholinesterase activity. Goldman et al. (1987) measured
RBC-AChE and plasma-ChE levels in rats following a single subcutaneous
injection of 1 μg/kg. RBC-AChE was depressed to a greater extent and for
a longer time period than was plasma-ChE, reportedly typical of some other
organophosphate anticholinesterase agents (Wills 1972). The greatest

depression of RBC-AChE (42%) occurred 2 hr after injection, remained depressed for 24 hr, returned toward control value over a period of 24 to 240 hr, and was 90% of the control value 240 hr after injection. The plasma-ChE was most severely depressed after 0.5 hr (51%), remained at this level for 2 hr and returned to 90% of the control level by 72 hr after injection.

In a subchronic toxicity study, Goldman et al. (1987) injected rats subcutaneously with 0.25, 1.0, or 4.0 μg/kg of VX daily for 30, 60, or 90 days. RBC-AChE activity was reduced in a dose-dependent manner at all time points and returned to control values when allowed a 30-day recovery period. Plasma-ChE levels were significantly depressed in the two higher dose groups at 30, 60, and 90 days and returned to control values after the 30-day recovery period. Overall there were no significant dose effects, but males exhibited a significantly higher level of enzyme inhibition than females at all time periods.

Although surviving animals recover from acute toxic effects of VX in about a week, recovery of RBC-AChE levels to normal requires two weeks or longer (Marzulli et al. 1960). This observation indicates that the recovery of cholinesterase in target tissues is more rapid than regeneration of RBC-AChE levels.

Two studies provide information on recovery processes in muscle and brain tissues. Goudou and Rieger (1983) studied the time course of AchE recovery in mouse muscle following a single intraperitoneal injection

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of VX. After inhibition, there was an initial (1 to 15 hr) rapid recovery of AChE (from about 20 to 60% of the control values), followed by a slow phase of return. After 3 days, the recovery was still incomplete, reaching 70-80% of control values. Recovery was attributed only to de novo AChE biosynthesis and not to spontaneous reactivation of phosphorylated enzyme.

Using a radioactive tracer, Goossens et al. (1984) followed the recovery of brain AChE activity in rats after inhibition by VX. The recovery process also proceeded in a biphasic manner, consisting of a rapid phase of about 30 min and a slower phase of about two days. Return to control levels occurred around the seventh day. In this study, the investigators suggested that a reactivation process occurred in at least a fraction of the brain enzyme during the initial rapid phase, and de novo biosynthesis of new active enzyme during the slow phase.

3.2.7 Miosis

Effects of organophosphates which do not require systemic absorption, such as miosis (pupillary constriction), may occur at exposure levels lower than those producing inhibition of blood cholinesterase and are generally noticed before the effects on other systems. Miosis, a sensation of pressure behind the eyes, headache, and conjunctival hyperemia are responses to localized exposure (Grob and Harvey 1953). According to NRC (1982), when applied to the conjunctiva, anti-cholinesterase agents produce miosis in a few minutes, reaching maximal constriction within

0.5 hr, and returning to normal within a few hours or days depending on the chemical and its concentration. In tests on human volunteers, unilateral miosis occurred as a result of vapors rising from a small amount (5 μg/kg) of liquid VX applied to the face (Sim 1962).

The miotic effects of systemic administration are not as predictable as those of local exposure, and either constriction or dilation of pupils may be seen. For example, Kimura et al. (1960) observed dilation of pupils in a volunteer injected intravenously with 0.225 μg/kg of VX. Miosis was not noted in subjects receiving intravenous or oral doses of VX in tests conducted on human volunteers by Sidell and Groff (1974).

McNamara et al. (1971, 1973) used miosis as an endpoint in determining the minimum-effect and no-effect levels of exposure to VX vapor in humans. The ECt_{50} for VX in man (0.09 mg-min/m^3) was extrapolated from data on rabbits with the assumption that man is twice as sensi-tive as rabbits (as is the case for GB-induced miosis). The no-effect level was derived by comparison of VX and GB ECt_{50} values for rabbits. The ECt_{50} value of VX for miosis in the rabbit is 0.17 mg-min/m^3, while that for GB is 4.36 mg-min/m^3. VX is much more efficient than GB in producing miosis during vapor exposure, probably because of its greater penetration of the surface of the eye. The Ct required to produce miosis may be as little as 1/25 that of GB. This same relationship was thought to be applicable to man, for whom the no-effect dose rate for GB was established as 0.5 mg-min/m^3. The no-effect dose rate for VX therefore was estimated as 0.02 mg-min/m^3 (see also Section 5.4.1).

Increasingly severe miosis would occur at exposures up to about 0.3 mg-min/m^3 which should produce maximal response.

More recent experimental data from subacute inhalation studies with VX showed that doses of 0.000005 to 0.004 mg/m^3 (6 hr/day for 5 or 10 days) produce a dose-related incidence of miosis in rodents and rabbits (Crook et al. 1983) (see also Section 3.2.8).

3.2.8   Minimum-Effect Levels

A percutaneous study by Sim (1962) showed that following a single skin application of 5 μg/kg of VX to the cheeks or ear lobe area, about half the subjects would experience moderately severe gastrointestinal symptoms (nausea and vomiting). The time of onset of symptoms ranged from 3 to 10 hr after treatment. Application to the ear lobe produced illness in the shortest time period (median 4 hr). The same dose applied to the face produced sufficient vapor to cause unilateral miosis in some individuals. Cholinesterase depression and incidence of symptoms were directly correlated when doses of VX ranging from 5 to 20 μg/kg were applied to the skin.

Subacute inhalation studies on mice, rats, guinea pigs, and rabbits showed that doses between 0.000005 and 0.0002 mg/m^3/day (6 hr/day) for 5 and 10 days would cause a slight to moderate RBC-AChE depression in the first week followed by gradual recovery, probably to baseline values

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(Crook et al. 1983). Higher doses produced a gradual increase in enzyme inhibition to the point where no recovery would occur. The average RBC-AChE depressions for all species tested was 77%, 55%, 23%, and 7% for 5-day exposures to 0.004, 0.0002, 0.00006, or 0.000005 mg/m^3, respectively. Exposure to the same concentrations for 10 days produced 78%, 46%, 14%, and 0% enzyme depression, respectively.

Exposure levels of 0.000005 to 0.0004 mg/m^3 for 5 to 10 days (6 hr/day, 5 days/week) also produced miosis (Crook et al. 1983). The response was dose-related, producing no effect (rats and guinea pigs) or only slight miosis (mice and rabbits) at the lowest dose level and significant effects at the highest level in at least three species (mouse, rat and rabbit). The average miotic responses (number of animals affected), for all animals tested was 75%, 43%, 13%, and 21% for 5-day exposures to 0.004, 0.0002, 0.00006, or 0.000005 mg/m^3, respectively. Exposure for 10 days produced miosis in 67%, 43%, 40%, and 15% of the animals tested at the four doses, respectively (Crook et al. 1983).

3.2.9 No-Effect Levels

According to McNamara et al. (1973), the estimated no-effect dose for VX-induced tremors in a 70-kg man, on the average, is 0.34 μg/kg (1.6 mg-min/m^3). This value was derived from human and animal data with GB which indicate that 4.0 mg-min/m^3 would produce no neuromuscular effects in man. Furthermore, inhaled VX at low dose levels was found to be about 2.3 times as toxic as GB in animals.

An intravenous injection of 0.1 $\mu g/kg$ into humans produced no detectable toxic effects, including inhibition of ChE (Kimura et al. 1960). The equivalent Ct was calculated to be 0.47 mg-min/m^3 (Carnes et al. 1986).

As discussed in Section 3.2.7, McNamara et al. (1971, 1973) used miosis as the endpoint in determining the minimum-effect and no-effect levels of VX in humans. The ECt_{50} of VX for miosis in man (0.09 mg-min/m^3) was extrapolated from data of locally applied GB on rabbits. By analogy from the GB dose-response rate in humans, the no-effect dose rate for VX-induced miosis was estimated as 0.02 mg-min/m^3.

3.3 CHRONIC TOXICITY

Most of the health effects studies on VX have dealt with acute toxicity of this chemical agent. With the potential for chronic exposure in the occupational setting, the possibility of adverse health effects induced by VX is of particular importance. However, very little evaluation of potential chronic effects from VX exposure has been performed. Available data are summarized as they relate to various chronic health endpoints.

A study was conducted by the National Research Council Panel on Anticholinesterase Agents (NRC 1982) to evaluate the possibility of long-term or delayed health effects of chemical agents (including VX) that were tested on military volunteers during the 1950s and 1960s. The study found

no evidence that any of the agents produced long-term adverse human health effects. However, the panel did not rule out the possibility that the chemical agents may have produced subtle changes of EEG, sleep pattern, and behavior that persisted for at least one year. Although delayed physiological effects and EEG changes have been attributed to organo-phosphate pesticide exposure, no data concerning brain dysfunction following exposure to VX have been found (Karczmar 1984).

3.3.1 Carcinogenicity

Carcinogenic and mutagenic effects are not generally associated with organophosphate nerve agents. Evidence from a study on the long-term health effects of nerve agents administered to military volunteers conducted 25 years ago support this view (NRC 1982). Carcinogenicity has never been attributed to VX in personnel working daily with this agent (McNamara et al. 1971). However, definitive studies on the carcinogenic potential of VX are lacking.

3.3.2 Genotoxicity

Mutagenicity studies may have relevance in predicting carcinogenic activity. The genotoxic potential of VX has been addressed in several studies conducted by Goldman et al. (1987). The studies included the Salmonella Ames test on five different revertant strains (TA1535, TA100, TA98, TA1537, and TA1538), the mitotic recombination assay in Saccharomy-ces cerevisiae, the mouse lymphoma assay, and a dominant lethal test in rats.

In the Ames assay, five concentrations ranging from 0.01 to 10 μg VX/plate were used with and without the addition of rat liver microsomal enzymes. The reversion rates were essentially the same for all microsomal preparations and did not differ from the spontaneous reversion rate (Goldman et al. 1987).

Three concentrations of VX (25, 50, or 100 μg/mL) were employed in the Saccharomyces cervisiae bioassay for the induction of mitotic recombinations. The tests were done with and without metabolic activation, and with negative and positive controls. There was no increase in recombigenic activity associated with VX exposure (Goldman et al. 1987).

In the mouse lymphoma assay, VX was evaluated at concentrations ranging from 1 to 100 μg VX/mL of solution. While the highest two dosage levels (50 and 100 μg/mL) showed a slight, but statistically insignificant increase in the number of forward mutations over control levels, this increase was not considered of biological significance given the characteristics of the mouse lymphoma assay. It is noteworthy that the higher concentrations employed in these studies exceeded the lethal doses for man by three to four orders of magnitude in terms of μg VX/mL of body fluid (Goldman et al. 1987).

Using a modified dominant lethal test in rats, Goldman et al. (1987) also investigated the potential mutagenic effects of VX on germinal cells in intact animals. Either or both sexes were exposed to doses of 0.5, 1, or 4 μg/kg of VX. Under the parameters used in this test (number of

implants, live fetuses, dead fetuses, resorptions, as well as lesions and defects), VX did not produce dominant lethal mutagenic effects.

In conjunction with studies on the subacute effects of low-level VX concentrations on laboratory animals, Crook et al. (1983) conducted genotoxicity studies which included the Ames assay, the micronucleus test, and the Drosophila sex-linked recessive lethal test. Negative results were reported for all three tests. In the Ames assay, five tester strains of Salmonella were exposed to VX concentrations ranging from 2.7×10^{-6} to 1093 μg/plate (the authors estimated that 2.7×10^{-2} μg/plate approximates the estimated LD_{50} in man). In the micronucleus test, mice were exposed to VX at concentrations of 0.0002 mg/m^3 or 0.000005 mg/m^3. Mutation tests using Drosophila melanogaster employed concentrations of 0.004 or 0.000005 mg VX/m^3.

3.3.3 Teratogenicity/Reproductive Effects

Certain organophosphate pesticides similar in structure to the nerve agents have been shown to produce abnormal fetal development in a number of mammalian species. For example, Wilson (1977) noted that malathion produces malformations and intrauterine death and resorptions in rats. However, definitive studies with VX on developing embryonic or fetal systems and on reproductive functions, and evidence derived from accidental exposure of female sheep to VX, have not demonstrated teratogenic or adverse reproductive effects.

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Goldman et al. (1987) evaluated the potential for VX to induce teratogenic effects in rats and rabbits, concentrating on frequency of external visceral and skeletal abnormalities, sex ratios, and possible adverse effects on body weight. VX administered to Sprague Dawley rats at doses up to 4.0 μg/kg (the highest tolerated level) by subcutaneous injection on days 6 to 15 of gestation produced no evidence of teratogenic effects. Similar studies with white Dutch rabbits exposed to the same VX concentrations on days 6 through 19 of gestation also did not reveal teratogenic activity.

In a three-generation reproductive study, Goldman et al. (1987) exposed Sprague Dawley rats to 0.25, 1, or 4 μg VX/kg. Toxicity indices examined included decreased fertility, premature delivery, pup survival, and size of offspring, as well as changes in reproductive behavior, clinical observations, and gross histopathology and necropsy. There were no adverse reproductive effects which could be attributed to VX exposure for any of the parameters studied.

Evidence relevant to teratogenic or reproductive effects of VX was also derived from exposure of sheep following the accidental release of VX during aerial testing in the Skull Valley, Utah, in 1968 (Van Kampen et al. 1969, 1970). Sheep were exposed to VX by ingestion of contaminated forage or direct contact to presumably high doses of VX. Approximately 4,500 of 6,278 affected sheep died. Cholinesterase activity was significantly depressed up to four months after the initial signs of poisoning occurred. However, examination of surviving ewes and offspring of ewes

that were pregnant at the time of exposure did not show adverse effects on reproductive capacity or fetal growth and development. No effects on reproductive capacity were found when exposed ewes were bred 5 to 6 months after exposure.

3.3.4 Delayed Neuropathy

Delayed neuropathic effects following acute and subchronic exposures to organophosphates have been reported by a number of investigators (see Johnson 1975a, 1975b, 1981, Wagner 1983 for reviews). The clinical syndrome, which in humans involves weakness and ataxia of the lower limbs and degeneration of some nerve fibers, may not be seen for up to 14 days following exposure. According to many investigators, delayed neuropathy results from direct cellular damage caused by the inactivation of a specific enzyme, neurotoxic esterase (NTE) but not of AChE. Consequently, NTE activity in the nervous system has been used as a predictive tool for estimating the neurotoxic potency of various nerve agents.

There is a marked species difference in the production of delayed neuropathy: most rodents do not develop neuropathy easily, and only in the hen and cat can a syndrome similar to that in humans be induced (Gordon et al. 1983, Johnson 1975a). Abou-Donia (1981) states that potent cholinesterase inhibitors may not be easily tested for their capacity to induce delayed neuropathy because rapid metabolism and severe acute reactions do not allow the accumulation of the agent at the target site, and this accumulation is required to cause the delayed effect. He also questions

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the validity of NTE inactivation as a proposed mechanism of action for delayed neuropathy.

Experimental evidence indicates that VX is a poor inhibitor of NTE and does not produce a delayed neuropathic response in animals. Vranken et al. (1982) studied the in vitro inhibition of hen brain NTE activity by the chemical agents tabun (GA), sarin (GB), soman (GD), and VX. All the agents studied inhibited NTE with the exception of VX. Gordon et al. (1983) estimated that doses of nerve agent that cause 70 to 80% inhibition of NTE are necessary to produce experimental neuropathy. They found that agent GB was three times as potent as their reference compound DFP (diisopropyl-phosphorofluoridate) in inhibiting NTE, while VX was more than 1,000 times less effective as an inhibitor. Because of the potency of GB in inhibiting NTE, it was tested in hens for delayed neuropathy. The lowest effective dose was 30 times the unprotected LD_{50}. The syndrome therefore is produced by giving very large doses in combination with protection from the acute effects of GB poisoning. The investigators indicate that with increasing levels of protection that can be achieved with drug treatment against the acute toxicity of anticholinesterases, consideration should be given to the possibility of delayed neuropathic symptoms in survivors of poisoning due to GB, GD, or GA, but not to VX.

Results of the recent in vivo study conducted by Goldman et al. (1987) confirms that VX does not cause delayed neuropathic effects in hens. Following treatment with atropine and 2-PAM to protect against acute toxicity, several strains of hens were injected subcutaneously with single doses of 10, 100, or 150 μg/kg of VX and observed for three weeks

after treatment. The highest dose administered was estimated as 5 to 10 times the lethal unprotected dose. There were no behavioral and histological effects characteristic of delayed neuropathy, nor was there a decrease in NTE activity of the brain. The investigators noted, however, that the possibility of other neuropathic or myopathic effects should not be ruled out.

A subsequent multiple-dose study conducted by Goldman et al. (1987) in which chickens were given subcutaneous injections of 40 μg/kg of VX for 90 days supports the conclusion that VX does not cause organophosphorus-induced delayed neuropathy. The design of the study included treatment with atropine to permit high doses of the agent to be used. Treatment for 90 days and beyond did not produce axonal or spinal cord lesions characteristic of delayed neuropathy in VX-exposed birds. However, analysis of plasma enzymes showed increased lactic dehydrogenase and creatine kinase activity, indicating that VX may cause tissue damage sufficient to bring about the release of cytoplasmic enzymes.

While the above cited studies indicate that VX does not produce delayed neuropathic effects, Olajos et al. (1986) noted that an intermediate in the synthesis of VX, O-ethyl-O'-(2-diisopropylaminoethyl)-methylphosphonite or QL, produced such effects in hens given a single oral dose of QL ranging from 635 to 6080 mg/kg. The hens were observed over a 24-day post-treatment period. Beginning on the sixth day after treatment and thereafter, the hens exhibited motor impairment. The principal neural lesion observed was multifocal nerve fiber degeneration of the peripheral nervous system.

3.3.5 Subchronic Toxicity

Goldman et al. (1987) analyzed the subchronic toxicity of VX by injecting Sprague Dawley rats subcutaneously with 0.25, 1, or 4 μg of VX/kg of body weight. The injections were given once per day, 5 days per week, for up to 90 days. Preliminary studies indicated that a dose of 4 μg/kg was the maximum dose which did not cause lethality. Animals in the highest dose group exhibited increased irritability and aggressiveness early in the study and decreased grooming and lethargy later in the study. The 1 μg/kg group also showed increased irritability later in the study. These behavioral effects appeared to be more pronounced in males. The main toxicological effect of VX was a decrease in body weight in the 4 μg/kg dose group. Animals of all dose groups exhibited significant dose-related decreases in RBC-AChE; plasma-ChE levels were significantly lowered in the two higher dose groups. The enzyme activities returned to normal when allowed a 30-day recovery period. The RBC-AChE levels following subcutaneous injection are presented in Table 6; also included are data for shorter treatment periods (7 and 14 days). There were no consistent effects attributable to VX exposure on organ weights, clinical chemistry parameters, or histopathology. From these studies it was concluded that at levels up to those associated with acute toxicity, repeated administration of VX to rats over a 90-day period produced no systemic toxicity.

TABLE 6. RBC-AChE ACTIVITY[a] IN RATS INJECTED SUBCUTANEOUSLY WITH REPEATED DOSES[b] OF VX

Time of Sacrifice	Sex	No.	VX Dose (μg/kg/day)			
			0.25	1.0	1.56	4.0
7 days	m	4	0.85 ± 0.06	–	0.46 ± 0.07	0.31 ± 0.03[c]
	f	4	0.90 ± 0.05	–	0.36 ± 0.09	0.34 ± 0.13[c]
14 days	m	4	0.72 ± 0.07	–	0.34 ± 0.12	0.29 ± 0.03[c]
	f	4	0.64 ± 0.12	–	0.28 ± 0.13	0.31 ± 0.08[c]
30 days	m	2	0.46 ± 0.04	0.22 ± 0.00	–	0.04 ± 0.02
	f	2	0.48 ± 0.06	0.20 ± 0.01	–	0.10 ± 0.05
60 days	m	2	0.33 ± 0.17	0.23 ± 0.06	–	0.14 ± 0.00
	f	2	0.53 ± 0.04	0.34 ± 0.02	–	0.23 ± 0.02
90 days	m	3	0.34 ± 0.02	0.37 ± 0.03	–	0.23 ± 0.08
	f	3	0.64 ± 0.13	0.51 ± 0.17	–	0.27 ± 0.07
90 days + recovery[d]	m	5	0.91 ± 0.07	0.88 ± 0.11	–	0.83 ± 0.11
	f	5	0.98 ± 0.12	0.93 ± 0.13	–	0.88 ± 0.16

Source: Goldman et al. 1987

a Data are expressed as mean ± SD as fraction of control values
b Dosing schedule once per day, five days per week
c Dose was 3.91 μg/kg/day
d Recovery period was 30 days

3.4 POTENTIAL FOR PERFORMANCE-DEGRADING EFFECTS

A factor relevant to developing an occupational standard for VX is the level of AChE inhibition above which performance-degrading effects could occur in occupationally exposed personnel. Based on an analysis of the responses of subjects who received a single percutaneous dose of VX, Sim and Stubbs (1960) indicated that no performance-degrading health effects are likely until the RBC-AChE activity is depressed to below 50% that of pre-exposure levels (Table 4). Sidell and Groff (1974) described the symptoms and ChE activity in man after intravenous administration of VX at doses ranging from 1.2 to 1.7 μg/kg (see also Section 3.2.4). One of the toxicity measures was performance. Most of the subjects had mild to moderate increase in heart rate and blood pressure during the first 3 hours after injection of VX; however, these values did not differ significantly from those of the control group. During the first hour only, the Number Facility Test scores were 71% for the 1.5 μg/kg treated group vs. 97% (p <0.01) for the control group. In the highest dose group, symptoms were maximal at one hour, coinciding with minimal ChE values.

Gage (1967) reports that surveys of normal unexposed populations have indicated that the coefficient of variation for the scatter of ChE activity ranges between 10 and 15% for red blood cells and between 15 and 25% for plasma, leading to the conclusion that an individual with a red cell activity 20% below the population average, or a plasma activity 33% below has probably been exposed to a ChE inhibitor. This calculation, however, does not take into account intra-individual variations of enzyme

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activity, i.e., a single determination on an individual with a naturally high or low activity when referred to the population average may lead to a faulty interpretation. He states that there is no general agreement on the degree of ChE inhibition which is likely to be associated with toxic manifestations, but suggests that toxic effects probably would not occur unless the RBC-AChE activity is depressed below 25% of normal (75% reduction in activity).

For occupational exposure to chemical agents, however, Gage (1967) suggests the removal of workers from employment when either RBC or plasma enzyme activity fall to 70% of normal (30% depression), so that further absorption will not lead to deterioration of health (not due to any risks of immediate toxic effects). He also suggests that when the measured blood enzyme activity returns to 80% of its normal value (20% depression), the worker may return to work. Similar measures are proposed by the World Health Organization (WHO 1975, as reported in Lauwerys 1983) for the protection of workers handling the organophosphate pesticide parathion. According to WHO, worker exposure should be discontinued when plasma-ChE activity is reduced to 50% of preexposure levels or when RBC-AChE activity is reduced to 70% of preexposure levels. Similar action levels were supported at a workshop on biological monitoring of persons exposed to ChE inhibitors (Zavon 1976). Because blood levels of cholinesterase vary considerably from individual to individual, preexposure baseline data are needed for comparison with postexposure levels.

3.5 TREATMENT OF VX POISONING

Numerous reports are available that describe therapeutic measures to counter VX toxicity. A review of some of the literature (Brown and Joffe 1961; Vick and Roberts 1975; Matsumara 1976; Meselson and Robinson 1980; Taylor 1980; Ellin 1981; Weger and Szinicz 1981; Harris and Stitcher 1983; Puu et al. 1986) indicates that the best treatment is a combination of atropine and an oxime such as pralidoxime or obidoxime. Treatment with the combination of atropine and an oxime is more effective than treatment with either one alone. It is thought that atropine blocks the action of acetylcholine which has accumulated to excess because of VX inhibiton of AChE; oximes reactivate inhibited ChE by dephosphorylation. The simultaneous action of these processes therefore would enhance recovery over the use of either alone. Pretreatment with carbamates such as pyridostigmine or physostigmine enhances the efficiency of atropine and oxime therapy, but the protective action of carbamates is not completely understood (Rickett et al. 1987). The two carbamates are reversible inhibitors of AChE, but also interact with the nicotinic receptor-channel complex. Physostigmine appears to act as a weak agonist, while pyrodistigmine is an open channel blocker (Albuquerque et al. 1984).

The reactivation of VX- and GB-inhibited AChE by pralidoxime has been studied in vivo by Sidell and Groff (1974). They found that the reactivation of the VX-inhibited enzyme is quicker than that for the GB-inhibited enzyme and that the dose of pralidoxime necessary for reactivation is smaller for VX than for GB. VX-inhibited AChE was amenable to

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reactivation for as long as 48 hr, with 70% reactivation by pralidoxime at all doses (2.5-31.6 mg/kg) and times (0.5 hr-48 hr) tested. About 10-20% of the VX-inhibited RBC-AChE was not reactivated by any dose of oxime at any level. The VX-inhibited enzyme "aged" more slowly than GB-inhibited enzyme. The rate of spontaneous reactivation decreases rapidly as the P-OR group increases in bulk. Thus, the rate of spontaneous reactivation is considerably slower when R is isopropyl (as in GB) than when R is ethyl (as in VX). Aging is due to the hydrolysis of these groups from the phosphorylated enzyme. Although VX is 3 to 4 times more potent than GB as an inhibitor of AChE, chances of recovery following treatment are much better in the case of VX.

4. CURRENT STANDARDS

Federal regulatory agencies have not developed regulatory standards for VX.

The Department of Defense (DOD) occupational exposure limit for VX is 0.00001 mg/m^3 (1 x 10^{-5} mg/m^3) for an 8-hr-time-weighted average (TWA) (DOD 1984). This occupational standard is based on recommendations by McNamara et al. (1973) who used miosis and RBC-AChE activity as their criteria for a no-effect level. For the general public, the DOD lists two exposure limits: 0.00001 mg/m^3, ceiling value, and 0.000003 mg/m^3 (3 x 10^{-6} mg/m^3) for a 72-hr TWA.

Public Law 91-121/441 (50 USC 1512) mandates that the Department of Health and Human Services (DHHS) review DOD plans for disposing of lethal chemical munitions and make recommendations to protect human health (DHHS 1987a). Based on a recent review of toxicity data, specifically human data on ChE inhibition, the Department of Health and Human Services, Centers for Disease Control, determined that human health will be adequately protected from the effects of VX vapor at the following levels: general population, 0.000003 mg/m^3 for a 72-hr TWA and workers, 0.00001 mg/m^3 for a 8-hr TWA. These levels contain a safety factor of about 1000 for the general population and 500 for workers (DHHS 1987a) and are the same as the limits developed by DOD.

The DOD source emission limit is 0.00003 mg/m^3 (3 x 10^{-5} mg/m^3) for a 1-hr TWA. According to DHHS (1987b), the Army has proposed a maximum allowable concentration of 3 x 10^{-4} mg/m^3 (reference not given). DHHS (1987b), in reviewing health and safety aspects of the demilitarization of VX, considers the DOD allowable stack emission levels to be more restrictive than limits set on health basis alone, and therefore recommends no changes in allowable concentrations.

The U.S. Army has set a maximum permissible water concentration for agents GA, GB, GD, and VX (sum total and individual levels) at 0.02 mg/L (USMERDC 1975).

5. BASIS FOR CURRENT OCCUPATIONAL EXPOSURE STANDARD FOR VX

5.1 BACKGROUND INFORMATION

The occupational exposure standard for VX currently used by the Department of Defense is based upon the recommendations of McNamara et al. (1973). McNamara et al. reviewed the available toxicological data to determine the minimal toxic effect level for exposure to VX vapor and concluded that toxicity thresholds could be identified by miosis and by the inhibition of acetylcholinesterase (AChE) in the blood. Miosis, the contraction of the pupil of the eye, was considered to be the lowest observable effect for direct contact with VX. Inhibition of blood-AChE was considered to be the lowest measurable effect following systemic absorption.

5.2 CURRENT EXPOSURE STANDARD BASED ON MIOSIS

5.2.1 Estimates of No-Effect Dose

McNamara et al. (1973) derived their miosis-based exposure standard from a comparison with toxicity data reviewed in an earlier study on the cholinesterase inhibitor GB (McNamara and Leitnaker 1971). They reported that for GB, the ECt_{50} values for miosis in humans and rabbits were 2-3 and 4.36 mg-min/m^3, respectively, showing that humans were about 2 times more sensitive to GB than rabbits. For VX, they reported an experimentally derived ECt_{50} of 0.17 mg-min/m^3 in rabbits, but no experimental data were available for VX-exposed humans. However, assuming that humans would be twice as sensitive as rabbits (as in the case for GB), they estimated that the ECt_{50} for VX-induced miosis in humans would

be 0.09 mg-min/m^3 (0.17/2). McNamara and Leitnaker (1971) had also

reported that the no-effect exposure for GB-induced miosis in humans was

equivalent to a Ct of 0.5 mg-min/m^3. Noting that in rabbits the ECt_{50}

for VX-induced miosis was about 25 times lower than that for GB (0.17

mg-min/m^3 versus 4.36 mg-min/m^3), they estimated that the no-effect

exposure for VX-induced miosis in humans would be 1/25 of the threshold

dose for GB-induced miosis. This procedure can be illustrated by the

following equations (where NOEL is the no-effect level for

miosis in humans):

$$VX \text{ NOEL} = GB \text{ NOEL} \times \frac{VX\text{-}ECt_{50} \text{ for miosis in rabbits}}{GB\text{-}ECt_{50} \text{ for miosis in rabbits}}$$

$$VX \text{ NOEL} = 0.5 \text{ mg-min/m}^3 \times \frac{0.17 \text{ mg-min/m}^3}{4.36 \text{ mg-min/m}^3}$$

$$VX \text{ NOEL} = 0.02 \text{ mg-min/m}^3$$

To estimate the no-effect dose for miosis for chronic exposures to

VX, McNamara et al. (1973) developed a mathematical model for dose-

effect accumulation (i.e., enzyme inhibition) based on rates of enzyme

recovery. Recovery from ChE inhibition was considered to be primarily a

function of the rate of reactivation of bound enzyme versus the rate of

aging of the enzyme-VX complex. In the case of erythrocytes, new AChE

would also be introduced into the blood with the formation of new red

blood cells.

McNamara et al. (1973), considered recovery of plasma-ChE to be a

"better measure of enzyme restoration" and a better index of recovery

from miosis, than recovery of RBC-AChE, since the latter is partially dependent on RBC turnover.

5.2.2 Models for Enzyme Recovery and Dose-Effect Accumulation

The model used by McNamara et al. (1973) for ChE recovery from VX is the same as that developed by McNamara et al. (1971) for the chemical agent GB. This model, which is based on a single acute dose, applies when one or more recovery mechanisms are operating independently, and can be expressed as the sum of n (2 or more) component exponential functions:

$$D(t) = D_0 \sum_{i=1}^{n} (f_i e^{-\lambda_i t}), \quad \text{when} \quad \sum_{i=1}^{n} f_i = 1$$

where: $D(t)$ = effect in units of dosage at time, t

D_0 = effect in units dosage present at time, $t = 0$

f_i = dimensionless fraction of dose effects disappearing at exponential rate of $-\lambda_i t$

t = time elapsed since dosing

λ_i = constant with reciprocal time units

where:

$$f_i(f_1, f_2, f_3 \ldots f_n) \quad \text{and} \quad \lambda_i(\lambda_1, \lambda_2, \lambda_3 \ldots \lambda_n)$$

are determined by standard curve-fitting techniques

Using the assumption that dose-effect accumulation is dependent on the rate of dosing and the rate of recovery, a general accumulation model can be expressed as:

$$E_t = D_d \sum_{i=1}^{n} \frac{[f_i(1-e^{-\lambda_i t})]}{\lambda_i}$$

where: E_t — effect in units of dosage, i.e., the acute dose to produce the effect

D_d — rate of dosing in units of dosage (as E) per unit of time

As t approaches infinity ($t \to \infty$), E approaches a constant value:

$$E = D_d \sum_{i=1}^{n} \left(\frac{f_i}{\lambda_i}\right)$$

For all three of the above equations, the simplest case is the one in which only one exponential component exists:

$$D = D_0 e^{-\lambda t}$$

$$E = \frac{D_d}{\lambda} (1 - e^{-\lambda t})$$

$$E = \frac{D_d}{\lambda}, \quad (t \to \infty)$$

McNamara et al. (1973) state that although the validity of the model cannot be proved, the model was shown to fit the available experimental data for GB (McNamara et al. 1971). The value of λ for recovery of VX-inactivated plasma-ChE, which was obtained by McNamara et al. from experimental data for six human subjects, was reported to be 0.40/day.

5.2.3 Derivation of the Current Exposure Standard

Applying the derived value of 0.02 mg-min/m^3 for the acute no-effect dose for miosis in humans, and the plasma-ChE recovery rate constant of 0.4 to their model for dose accumulation, McNamara et al. (1973) calculated the maximum safe dose for chronic exposures:

$$D_d \quad = \quad \lambda \times E$$
$$D_d \quad = \quad 0.4 \times 0.02 \text{ mg-min/m}^3$$
$$D_d \quad = \quad 0.008 \text{ mg-min/m}^3$$

This dose equates to an exposure of 0.000017 mg/m^3, 8 hours per day, every day for an unlimited period of time. McNamara et al. proposed that the occupational exposure standard for VX for workers not using eye protectors be 0.00001 mg/m^3.

Since the methodology used by McNamara et al. (1973) is based on extrapolations from the miosis data for GB, the exposure standard for VX can, in simplified terms, be approximated from the GB standard. Since the ECt_{50} dose for VX-induced miosis (in rabbits) was about 1/25 of the GB dose, while the recovery rate constant for VX was 4 times greater than that for GB (0.4 versus 0.1 for plasma-ChE), the overall net difference can be expressed as 4/25. Consequently, the VX standard can be estimated to be about 4/25 of the GB standard (0.0001 mg/m^3), or 0.000016 mg/m^3.

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5.3 EXPOSURE STANDARD BASED ON RBC-AChE INHIBITION

Since the eyes of workers can be protected against the effects of VX vapors by the use of occlusive goggles, McNamara et al. (1973) also calculated occupational exposure standards for such protected workers based on the systemic absorption of VX and the no detectable level of depression of erythrocyte-AChE.

5.3.1 Estimated No-Effect Level for RBC-AChE Inhibition

McNamara et al. (1973) derived their acute no-effect dose for VX-induced RBC-AChE inhibition from statistical considerations of the lowest dose that would not yield measured values of enzyme inhibition outside the 99% confidence range for unexposed individuals. Based on the assumption that random fluctuations in measured values of enzyme inhibition would result in a coefficient of variation of about 3%, as determined from differences between duplicates, the 95% confidence interval was reported as being ± 8.4% from the baseline value, and the 99% confidence interval as being ± 12.5% from the baseline value. From the reported ED_{50} of 1.0 μg/kg for VX inhibited RBC-AChE in humans, and assuming a pseudo first-order relationship, McNamara et al. calculated that the mean expected enzyme inhibition from 0.1 μg/kg would be 6.7%, a level which would allow for a high probability that some measurements would still indicate a significant level of enzyme inhibition (i.e, >12.5%). Assuming that 1/3 of the 0.1 μg/kg dose would result in 1/3 the effect, i.e., 2.3% mean enzyme inhibition, McNamara et al. predicted that there would be virtually no chance of detecting a significant level of enzyme inhibition (>12.5%); therefore, 0.03 μg/kg was taken as the no-effect level for a single acute exposure.

To estimate the no-effect dosing rate for chronic exposures to VX, McNamara et al. (1973) used their mathematical model for dose-effect accumulation based on rates of RBC-AChE recovery.

5.3.2 Models for RBC-AChE Recovery and Dose-Effect Accumulation

The prediction of dose-effect accumulation for RBC-AChE depression as used by McNamara et al. is based on enzyme recovery from a single acute dose. Recovery is defined by McNamara et al. as a multicomponent process described by the equation:

$$D_t \simeq D_0 \, [f_1 e^{-\lambda t} + f_2 (1 - t/\tau)]$$

where:

> f_1 = fraction recovering by a first-order process
> f_2 = fraction recovering by a zero-order process
> $f_1 + f_2 = 1$
>
> τ = life span of RBC (about 120 days)

The exact equation is written as:

$$D_t = D_0 \, [f_1 e^{-\lambda t} - f_2 (1 - t/\tau) e^{-\lambda_1 t} + f_2 (1 - t/\tau)]$$

showing that there are two exponential components and one linear one. According to McNamara et al., if the agent is delivered at a constant rate, D_d, the accumulation of the effective dose for RBC-AChE depression can be predicted by:

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$$E_t = D_d \frac{1}{\tau} \left[f_1 \frac{e^{-\lambda t}}{\lambda} - f_1(1-e^{-\lambda t})\left(\frac{1}{\lambda^2 t} - \frac{\tau}{\lambda t}\right) + f_2\left(\tau - \frac{t}{2}\right) \right]$$

where $\tau \geq t$ and E_t is the effective dose for those red blood cells aged τ or less time, t. E_t reaches a maximum at $\tau = t$:

$$E_{max} = E_\tau = D_d\left[\frac{f_2 \tau}{2} + \frac{f_1}{\lambda} - \frac{f_1(1-e^{-\lambda \tau})}{\lambda^2 \tau}\right]$$

McNamara et al. note that because the life span of red blood cells is 120 days, a steady state for E is reached at E_{120}. Derivation of the equation shows that:

$$f_1 = \frac{\lambda_2}{\lambda}$$

$$f_2 = \frac{\lambda_1}{\lambda}$$

where:

λ_1 = the first order rate constant for aging

λ_2 = the first order rate constant for dephosphorylation

$\lambda = \lambda_1 + \lambda_2$

McNamara et al. (1973) reported that estimates of λ_1 and λ_2 could be derived from the studies of Berry and Davis (1966) and Harris (unpublished data). These values were given as follows:

$$\lambda_1 = 0.278/\text{day}$$

$$\lambda_2 = 0.693/\text{day}$$

therefore:
$$f_1 - 0.714$$
$$f_2 - 0.286$$
and:
$$\lambda - 0.971/\text{day}$$

Inserting these values into the equation for E_{max} results in:

$$E_{max} - 17.89\ D_d$$

The model therefore predicts that under steady-state conditions, a constant dose of VX will be equivalent to about 1/18 of an acute dose in terms of dose-effect accumulation.

5.3.3 Derivation of the Current Exposure Standard

Applying the derived value of 0.03 μg/kg for the acute no-effect dose for RBC-AChE inhibition in humans, and the factor of 1/18 to their model for dose-effect accumulation, McNamara et al. (1973) calculated the maximum safe dose for chronic exposures:

$$D_d - 1/18 \times E$$
$$D_d - 1/18 \times 0.03\ \mu\text{g/kg}$$
$$D_d - 0.00168\ \mu\text{g/kg}$$

This no-effect daily dose is equivalent to 0.00783 mg-min/m^3, or 0.0000163 mg/m^3 for a daily 8-hr exposure (based on a breathing rate of 0.015 m^3/min for a moderately active 70 kg man).

5.4 EVALUATION OF THE METHODOLOGIES USED FOR THE CURRENT STANDARD

5.4.1 Standard Based on Miosis

In the absence of human data, McNamara et al. (1973) estimated the no-effect level for miosis in humans by assuming that the ratio of the no-effect doses for VX and GB would be equivalent to the ratio of the ECt_{50} doses for VX and GB in rabbits. A more reliable estimate of the no-effect level would have been obtained if human data for the ECt_{50}s had been used for establishing the relative potency of GB and VX for inducing miosis; however, human data were not available, and in fact the reported human ECt_{50} of 0.09 mg-min/m^3 was derived from the GB data. No human experimental data have since become available to verify the estimated acute no-effect value of 0.02 mg-min/m^3.

McNamara et al. (1973) used plasma-ChE inhibition to determine rates of recovery from miosis, stating that in studies on GB, plasma-ChE changes could be more closely correlated with changes in brain-ChE activity, and, therefore, would more truly reflect recovery from toxi-city. Also supporting this approach is a report by Sidell (1973) which indicated that in three of five patients accidentally exposed to GB, pupillary recovery paralleled plasma-ChE recovery. For GB, plasma-ChE recovery takes a shorter time than RBC-AChE recovery and follows a single component, first-order process. For VX, the initial rates of recovery for plasma-ChE and RBC-AChE are similar; however, there is a prolonged second phase in the recovery of RBC-AChE. The slow phase of recovery can be correlated with the replacement of erythrocytes con-taining VX-bound AChE that had undergone aging and which was therefore

not subject to spontaneous reactivation. Theoretically, pupillary recovery from the effects of VX would likely parallel the initial rapid recovery phase of RBC-AChE activity.

McNamara et al. (1973) estimated the plasma-ChE recovery rate constant, λ, from data for seven human subjects. The half-times for plasma-ChE recovery for these individuals ranged from 0.4 to 3.0 days and the calculated individual λ values ranged from 0.23 to 1.73. The mean half-time was 1.75 days and the mean λ 0.60/day. McNamara et al. (1973) selected as their final λ, 0.40/day, the value corresponding to the mean half-time. If the lowest reported λ of 0.23 had been used, the chronic no-effect dose would be about 0.004 mg-min/m^3 instead of 0.008 mg-min/m^3. If the mean λ of 0.60/day had been used, a value similar to the recovery rate constant for the rapid phase of RBC-AChE recovery, then the final chronic no-effect level would be 0.012 mg-min/m^3.

5.4.2 Standard Based on RBC-AChE Inhibition

McNamara et al. (1973) used RBC-AChE inhibition, rather than plasma-ChE inhibition, as their endpoint for calculating an occupational exposure standard for systemically absorbed VX. Unlike plasma-ChE, RBC-AChE (acetylcholinesterase) is identical to the enzyme found in the nervous tissue, and, there is a better correlation between VX-induced toxicity and RBC-AChE inhibition than plasma-ChE inhibition. Furthermore, total recovery from RBC-AChE inhibition takes longer than that for plasma-ChE inhibition; therefore, the calculation of a chronic no-effect level on the basis on RBC-AChE activity provides for a more conservative final value.

Several studies support the use of RBC-AChE for setting exposure standards. Grob and Harvey (1958) reported that in the case of GB, plasma-ChE was slightly less sensitive than the cholinesterase present in red blood cells, muscle, and brain. In studies on humans intravenously injected with VX, Sidell and Groff (1974), found that VX appeared to inhibit RBC-AChE preferentially. Doses in the range of 1.2 to 1.7 ug/kg, which resulted in symptoms of toxicity in 11 of 18 individuals, caused 50 to 80% reductions in RBC-AChE, but never more than a 20% reduction in plasma-ChE.

The exposure standards for VX calculated by McNamara et al. (1973) were based on the maximum level of exposure resulting in no-effect on RBC-AChE, i.e., the level producing no incidents of enzyme inhibition outside the 99% confidence range (± 12.5% AChE depression) for random fluctuations in a normal unexposed population. This defines the no-effect level in terms of the highest value that statistically results in no significant measurable enzyme inhibition, and also implies that any drop in RBC-AChE activity should be avoided because of possible toxic effects. This approach avoids the question as to whether an exposure standard should be based on a no-effect level or on the maximum exposure level resulting in no observable adverse effects. Most experimental data indicate that for acute exposures, adverse toxic effects caused by cholinesterase inhibitors occur only when RBC-AChE activity is reduced 40% or more. Furthermore, regulatory agencies have generally adopted a level of 30% RBC-AChE inhibition as an action level for preventing possible toxic effects. Therefore, in terms of current methodologies for regulating organophosphate cholinesterase inhibitors, a RBC-AChE depres-

sion of 30% might be considered a lowest-observable-adverse effect level (LOAEL). A no-observable-adverse-effect level (NOAEL) might be then selected at about 15% inhibition which would allow for the normal range in variation of measured RBC-AChE values (12.5% at the 99% confidence level, as calculated by McNamara et al.).

An important component of the methodology of McNamara et al. (1973) was the calculation of the adjustment factor by which their derived acute "no-effect" dose would be reduced to avoid potential cumulative toxic effects caused by dose-effect accumulation (i.e., the increase in the amount of AChE inhibited) during low level chronic exposures. The basic assumption for this calculation is that recovery from toxic effects parallels recovery from enzyme inhibition. For VX, McNamara et al. (1973), reported that, in terms of total enzyme recovery, plasma-ChE recovery was faster than RBC-AChE recovery. The latter was considered to be a combined zero-order process and a pseudo-first-order process, reflecting both spontaneous enzyme recovery and RBC turnover. However, for the first phase of recovery which would primarily be enzyme reactivation due to dephosphorylation, RBC-AChE recovery may be as fast or faster than plasma-ChE. From the data of Berry and Davis (1966), McNamara et al. (1973) reported an RBC-AChE dephosphorylation rate constant of 0.693/day whereas that for plasma-ChE was estimated to be 0.3/day. Furthermore, according to Sidell and Groff (1974), the VX-acetylcholinesterase complex may not "age" significantly over the initial 24-48 hr period, thus allowing a greater amount of reactivation. Sidell and Groff (1974) reported that VX-inhibited RBC-AChE initially reactivates spontaneously at a fast rate (this was estimated from the

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data depicted graphically by Sidell and Groff to amount to about 1% of the baseline value per hour).

The basic assumption of McNamara et al. (1973) that recovery from the toxic effects of VX would parallel recovery from inhibition of cholinesterase in the blood does not agree with some experimental data that show that low-level chronic exposure conditions can result in blood-ChE levels substantially below pre-exposure levels (i.e, >50%) without outward signs or symptoms of toxicity. This suggests that either the nervous system is less susceptible to low level chronic exposures, possibly due to the buffering action of blood-ChE, and/or that recovery of nervous system AChE occurs at a faster rate than that for the blood, and particularly for RBC-AChE. It would be expected that the rates of nervous system AChE dephosphorylation and aging should be similar to those for RBC-AChE. Rates of AChE synthesis might be expected to be higher in the nervous system; however, there are no experimental data to determine this. Thus, the dose-effect accumulation factor of 17.89 calculated by McNamara et al. (1973) on the basis of total RBC-AChE recovery may be overly conservative and the actual rate of recovery may parallel the initial rate of enzyme dephosphorylation in the erythrocytes. The recovery rate constant for RBC-AChE dephosphorylation was reported to be 0.693/day.

The method of McNamara et al. (1973) is also conservative in that the dose-effect accumulation factor is based on a model for continuous exposure whereas under actual conditions 8-hr exposures would be separated by 12-hr non-exposure periods with one 48-hr nonexposure period every five days. Thus, a model predicting enzyme recovery (either to a

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no-effect level, or to a NOAEL) for intermittent dosing would be more appropriate for use in calculating a dose-effect accumulation factor. Therefore, the value of 17.89 might be an upper limit for such an adjustment factor.

6. DERIVATION OF AN OCCUPATIONAL EXPOSURE STANDARD FOR VX

6.1 INTRODUCTION

In developing an occupational exposure standard for a chemical tox-
icant, the most important information that must first be derived from
experimental or epidemiological studies is the maximum exposure level
resulting in no adverse effects. This information can be obtained from
studies on humans or laboratory animals. For the chemical agent VX,
there is substantial evidence that miosis and the inhibition of blood
acetylcholinesterase (AChE) are the most sensitive measurable physiolo-
gical effects. In occupational settings direct contact of VX with the
eyes of workers would be avoided by the use of eye protectors such as
goggles, therefore, the inhibition of blood-AChE would be the most
appropriate parameter to use for limiting exposures to VX. Experimental
data for VX-induced AChE inhibition are available for both humans and
animals. For comparative purposes, both sets of data will be used to
derive an occupational exposure standard.

6.2 STANDARD BASED ON HUMAN DATA

The human data available for calculating an occupational exposure
standard for VX are limited to those derived from acute exposure studies
using single and multiple intravenous doses. In one study, Kimura et
al. (1960) found that an intravenous dose of 0.1 μg/kg produced no
measurable inhibition of RBC-AChE. In another study, Sidell and Groff
(1974), reported that single iv doses of 1.2 to 1.7 μg/kg resulted in
dose-dependant decreases in RBC-AChE activity, as expressed by the

following relationship (see Figure 1):

$$y = 57.6 - 188.7 \log x \quad (r = -0.71)$$

where: x = intravenous dose in $\mu g/kg$
 y = RBC-AChE activity (as % of baseline)

Using this formula, it can be calculated that the iv doses required to produce RBC-AChE depressions of 5, 15, 25 and 30% (95, 85, 75, and 70% of baseline) would be 0.63, 0.72, 0.81, and 0.86 $\mu g/kg$, respectively. Sidell and Groff (1974) also reported that in three unexposed individuals AChE activity fluctuated by not more than 5% around baseline values. In other studies fluctuations around baseline values in non-exposed individuals were generally not more than 15%. This corresponds fairly well with the calculation by McNamara et al. (1973) that for a coefficient of variation between duplicates of 3%, the random fluctuation of measured values would be within 12.5% of the baseline value 99% of the time; therefore, the smallest change in RBC-AChE activity which could be correlated with exposure to VX would be $\geq 12.5\%$.

The selection of a particular level of RBC-AChE inhibition as a measure of a lowest-observable-adverse effect level (LOAEL) or as a no-observable-adverse effect level (NOAEL), is difficult because of limitations in the available data. In some individuals, substantial reductions in RBC-AChE activity can occur without evidence of toxic effects. Sidell and Groff (1974) reported that 6 of 18 individuals treated with VX were asymptomatic even though their RBC-AChE levels were reduced to 14-44% of the baseline values. However, in most clinical studies it has been found that for single acute exposures to VX, symptoms of toxicity

will appear only when RBC-AChE activity is reduced 40% or more ($\leq$60% of baseline value). For exposures to the other cholinesterase inhibitors, a 30% reduction in enzyme activity has been recommended as the action level for removing workers from exposure (World Health Organization 1967; Zavon 1976). In the absence of more specific experimental data, a 30% level of AChE inhibition can be treated as a potential LOAEL for VX. Any level of enzyme inhibition less than 30% would probably have no adverse effects. A NOAEL might be selected at 15% (85% of baseline) to allow for a 15% variability in individual responses (which would include the 12.5% range in measured values at the 99% confidence level).

From the data of Sidell and Groff (1974), a 15% reduction in RBC-AChE activity corresponds to an iv dose of 0.72 μg/kg. An occupational exposure standard can then be calculated by applying the generalized formula for human parenteral data (see Opresko 1987, for derivation of equation and selection of parameter values):

$$C_a = \frac{NOAEL_{i.v.}}{[RESP_{occup} \times EXP_{occup} \times F_p]} \times bw \times \frac{1}{Uncertainty\ factors}$$

where:

C_a = Concentration in workplace air

$NOAEL_{i.v.}$ = No-Observed-Adverse-Effect Level (intravenous)
= 0.72 μg/kg

$RESP_{occup}$ = 43 L/min (breathing volume)

EXP_{occup} = 480 min (exposure period)

F_p = 0.80 (pulmonary availability)

bw = 70 kg (standard human body weight)

Uncertainty Factors = UF_1 x UF_2

UF_1 = 10 (to adjust for potential individual variability in sensitivity to VX)

UF_2 = 18 [to adjust for possible cumulative effects at low exposure levels, based on the model of McNamara et al. (1973)]

then:

$$C_a = \frac{0.72 \ \mu g/kg}{43 \ L/min \ x \ 480 \ min \ x \ 0.8} \ x \ 70 \ kg \ x \ \frac{1}{10 \ x \ 18}$$

C_a = 0.000017 $\mu g/L$

C_a = 0.000017 mg/m^3

Included in this calculation is a standardized pulmonary adjustment factor (F_p) of 0.8 which takes into account the presence of dead air spaces in the respiratory tract and the resulting reduction in aveolar availability. The F_p of 0.8 is used as a default value in the absence of experimental data to the contrary. In the case of VX, systemic absorption is likely to occur throughout the respiratory tract, and therefore, the F_p may actually be close to 1.0. Using a F_ρ of 1.0 in the equation would result in a final C_a of 0.000013.

Two uncertainty or safety factors are used in the above calcula-tion; one allowing for a potential ten-fold greater sensitivity for some individuals exposed to VX, and the other lowering the permissible expo-sure by a factor of 1/18 to allow for dose-effect accumulation. There are no experimental data concerning the range of individual sensitivity to the effects of VX. In an occupational setting, workers would be pretested for blood-AChE activity and only individuals with normal levels would be allowed in areas of potential VX exposure. Although

this suggests that the uncertainty factor of 10 for individual sensi- tivity to VX may be unnecessary in these situations, there are no exper- imental data to show that normal preexposure levels of RBC-AChE would preclude significant differences between individuals in the effects of VX on the nervous system. Further experimental data are needed to evaluate this possibility.

The factor of 1/18 which was used to ,.,t for potential cumula- tive effects is the same as that calculated by McNamara et al. (1973). The model from which this value was derived was based on continuous exposures. A model based on intermittent exposures would likely result in a higher final C_a. However, a value of 1/18 is not inconsistent with the available animal data which indicate that the effective dose for 15% RBC-AChE inhibition is about 1/24 of the acute dose for the same level of AChE inhibition (see Section 6.3).

The calculation given above represents exposure by inhalation only. According to the data of Cresthull et al. (1963), about 5% of the VX vapor could be absorbed through the skin. This additional exposure would have only a minor effect on the exposure standard, reducing the final value by 0.000001 mg/m^3.

6.3 STANDARD BASED ON ANIMAL DATA

Both acute and subchronic toxicity data are available for deter- mining the effects of VX on RBC-AChE activity in rats (Goldman et al. 1987). The effects of single subcutaneous doses are shown in Table 7 and plotted in Figure 2.
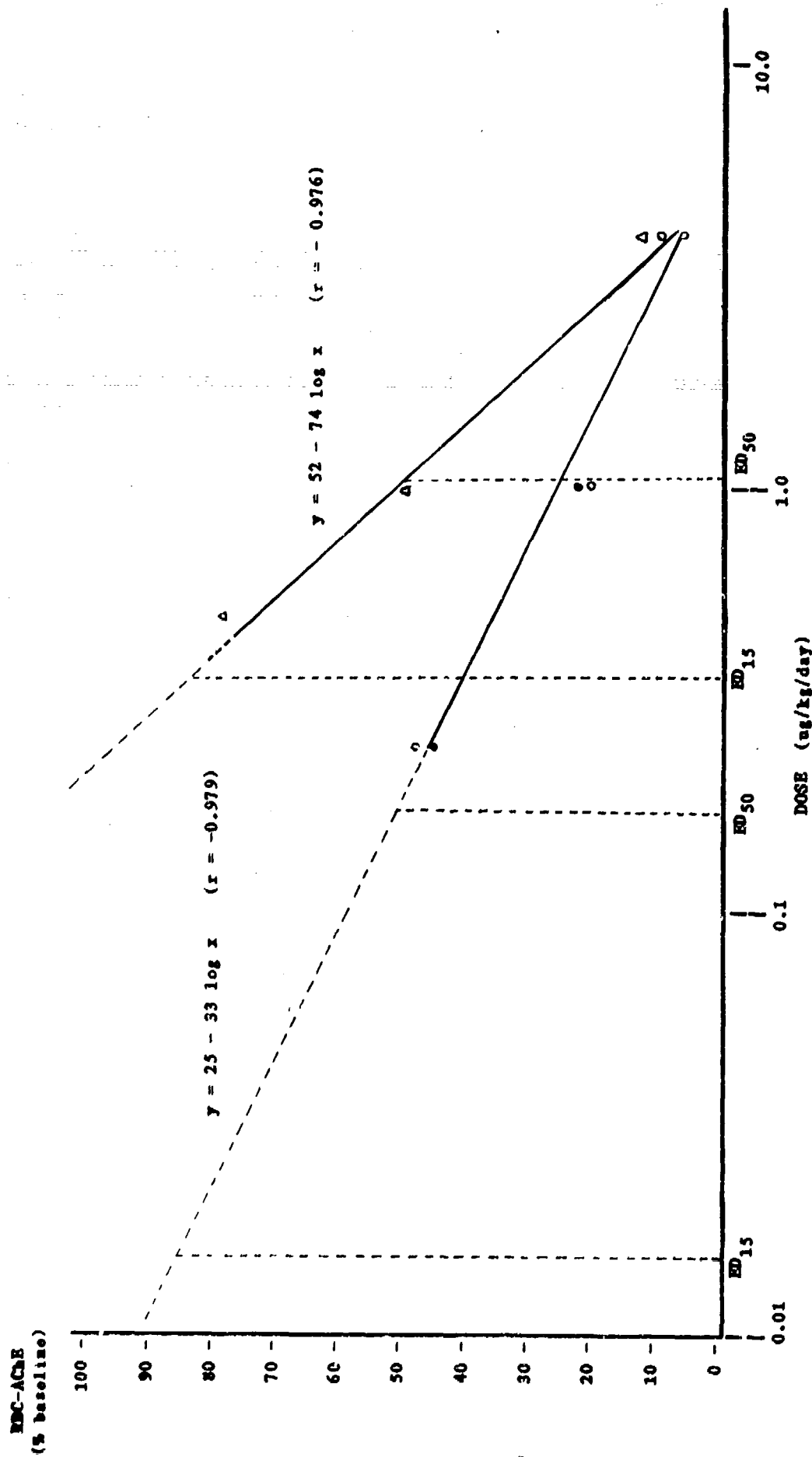
79

EBC-AChE
(% baseline)

$y = 52 - 74 \log x \quad (r = -0.976)$

$y = 25 - 33 \log x \quad (r = -0.979)$

DOSE (ug/kg/day)

Figure 2. Single acute ($\triangle$) and chronic (once per day, 5 days per week for 30 days in males (.) and females (o)) subcutaneous exposure of rats to VX versus measured EBC-AChE activity (as percent of baseline). (Derived from data reported in Goldman et al. 1987)

TABLE 7. RBC-AChE ACTIVITY[a] IN RATS INJECTED
WITH SINGLE SUBCUTANEOUS DOSES OF VX

Dose (μg/kg)	RBC-AChE Activity (% of control)
0 (control)	100
0.5	78 ± 15
1.0	50 ± 9
2.0	21 ± 3
4.0	13 ± 8

Source: Goldman et al. 1987

[a] Data expressed as mean ± S.D. (n = 4).

The data can be expressed by the following relationship:

$$y = 52 - 74 \log x \quad (r = -0.976)$$

where: x = subcutaneous dose in μg/kg
y = RBC-AChE activity (as % of baseline)

In subchronic bioassays, rats received daily subcutaneous injections of VX five days per week for up to 90 days. In one series of tests the administered doses were 0.25, 0.63, 1.56, and 3.91 μg/kg and RBC-AChE activity was monitored at 7 and 14 days. In another series of tests the administered doses were 0.25, 1.0, or 4.0 μg/kg per day and RBC-AChE activity was monitored at 30, 60, and 90 days. The combined data for four dose levels are given in Table 6 and plotted in Figure 3. The data reveal that there were cumulative effects (i.e, increased enzyme inhibition) over the first 30 days of the test period. However, at 60 days and 90 days there was a slight decrease in effect for all but one of the tests (males exposed to 0.25 μg/kg showed a slightly greater
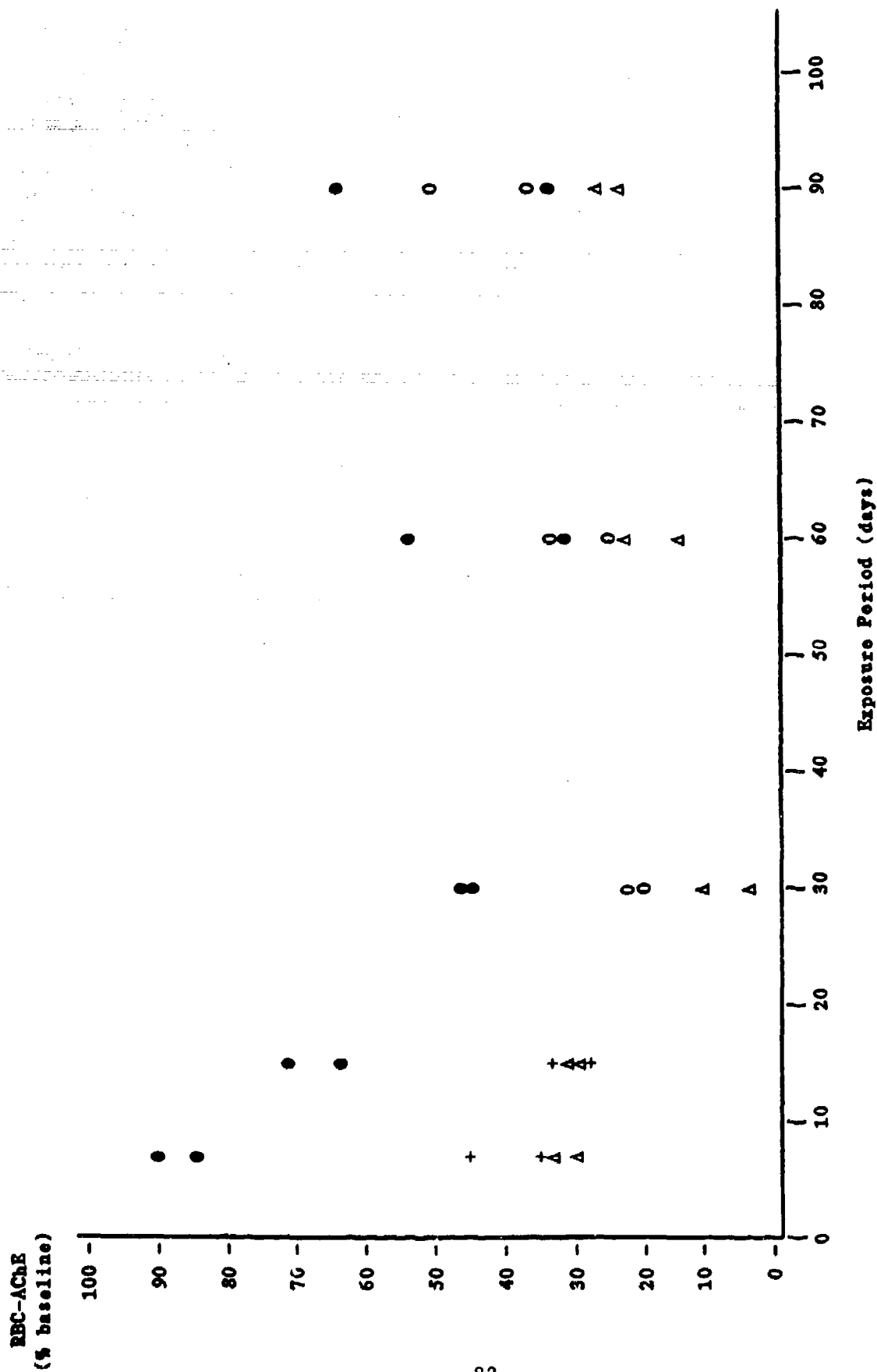
Figure 3. Effect of exposure period on RBC-AChE inhibition (% of baseline) in rats exposed subcutaneously to VX at dose levels of 0.25 ug/kg (●), 1.0 ug/kg (○), 1.56 ug/kg (+), and 4.0 ug VX/kg (△), daily, five days per week for up to 90 days. (Derived from data reported in Goldman et al. 1987)

level of enzyme inhibition than that occurring at 30 days, and this remained about the same at 90 days). Overall, the results of the Goldman studies indicate that for a dosing schedule of once per day for five days per week, the cumulative effect probably reaches a maximum value between 30 and 60 days. In the absence of data points between 30 and 60 days, the values at 30 days will be used as a measure of the maximum cumulative effect. These data points are plotted in Figure 2, and can be expressed by the following relationship:

$$y = 25 - 33 \log x \quad (r = -0.979)$$

where: x = dose in μg/kg
y = RBC-AChE activity (as percent of baseline)

The ED_{50} for AChE depression at 30 days is approximately 0.17 μg/kg. In contrast, the ED_{50} for a single subcutaneous dose is about 1.0 μg/kg (Fig. 2). As noted above, the difference between acute and chronic effective doses decreases as the dose level increases.

Extrapolation of the data of Goldman et al. (1987) indicates that the chronic dose producing a 15% depression of RBC-AChE after 30 days would be equivalent to about 0.015 μg/kg (Fig. 2). Using this value as a NOAEL, an occupational exposure standard can be calculated from the following formula (see Opresko 1987, for derivation of equation and selection of parameter values):

$$C_a = \frac{NOAEL_{sc}}{RESP_{occup} \times EXP_{occup} \times F_p} \times bw_h \times \frac{1}{(70/bw_a)^{1/3}} \times \frac{1}{\text{Uncertainty factors}}$$

where:

C_a = Concentration in workplace air

$NOAEL_{sc}$ = No-Observed-Adverse-Effect Level (subcutaneous)

= 0.015 μg/kg/day

$RESP_{occup}$ = 43 L/min (occupational respiratory volume)

EXP_{occup} = 480 min (occupational exposure time)

bw_h = 70 kg (standard human body weight)

bw_a = 0.4 kg (animal body weight)

$\dfrac{1}{(70/bw_a)^{1/3}}$ = 0.179 (interspecies adjustment factor for body size differences)

F_p = 0.8 (pulmonary availability factor)

Uncertainty Factor = 10 (to allow for intraspecies variability)

then:

$$C_a = \frac{0.015 \ \mu g/kg}{43L/min \times 480 \ min \times 0.8} \times 70 \ kg \times 0.179 \times \frac{1}{10}$$

C_a = 0.0000011 μg/L

C_a = 0.0000011 mg/m^3

The above calculation assumes that 100% of the subcutaneous dose would be absorbed systematically; however, a comparison of lethality data (see Table 2) suggests that a subcutaneous dose would produce one-half the lethal effect of an equivalent iv dose. If this also applies to non-lethal effects such as RBC-AChE inhibition, then the effective systemic dose at the NOAEL would be one-half of the applied dose, and therefore, the final calculated C_a would be one-half of that given above. As in the case of the calculations based on human dat·, the final value of C_a would also be decreased slightly if the assumption is

made that availability and absorption through the respiratory tract (F_p) in humans is 100% rather than 80%. The use of both modified assumptions would result in a final C_a of 0.0000005 mg/m^3.

6.4 COMPARISON OF CURRENT AND PROPOSED STANDARDS

In this report, an occupational exposure standard for VX, as based on human data, was calculated to be 0.000013 mg/m^3 for an 8-hr time-weighted average. A standard based on animal data was calculated to fall in the range of 0.0000005 to 0.0000011 mg/m^3, depending on the asumptions used. The smaller values derived from the animal data indicates that rats are much more sensitive to VX than humans. This difference in sensitivity can be seen by comparing the ED_{50} values for RBC-AChE inhibition between the two species. If the rat ED_{50} value of 1.0 μg/kg is used to derive an equivalent human dose (using 0.179 as an adjustment for body size), then the predicted human ED_{50} would be 0.18 μg/kg; however, clinical studies have shown that the human ED_{50} is about 1.0 μg/kg, indicating a substantially higher tolerance to VX. This may be explained in part by differences in blood-AChE activity between the two species. In rats RBC-AChE activity has been reported to be about 1/8 that of humans (Table 3); therefore, in rats the same dose would produce 8 times the effect.

The current DOD occupational exposure standard for VX is 0.00001 mg/m^3 for an 8-hr time weighted average. The standard calculated in this report from human data is 0.000013 mg/m^3. The DOD standard is based on the work of McNamara et al. (1973) who used statistical considerations for selecting 0.03 μg/kg as the no-effect dose for RBC-AChE

inhibition (based on detecting no significant enzyme depression outside the 99% confidence range for normal variability of measured values, i.e., > 12.5%). In contrast, in this report the experimental data of Sidell and Groff (1974) were used to derive the correlation between dose and enzyme inhibition. Because the available experimental data indicate that RBC-AChE inhibition of less than 30% would not result in adverse effects, 30% inhibition was selected as the LOAEL and 15% as the NOAEL. The latter value is equivalent to a dose of 0.72 μg/kg which is about 20-fold higher than the endpoint used by McNamara et al.

The dose-effect accumulation factor used in this report is 18, the same as that derived by McNamara et al. (1973). Animal data suggest that this is probably a reasonable value for extrapolating from acute to chronic exposures.

In the standard methodology used in this report an intraspecies uncertainty factor of 10 is used to allow for the possibility of extreme sensitivity of certain individuals to VX. This uncertainty factor was not used by McNamara et al. (1973).

In spite of the differences between the endpoints and the safety factors used in this report and those used by McNamara et al. (1973), the final calculated occupational exposure standards are almost identical, and, therefore, the current standard of 0.00001 mg/m^3 is considered to be valid.

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